GENE ANALYSIS BY THE PARTITIONING METHOD WHEN INTERACTIONS OF GENES ARE INVOLVED

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Introduction

POWERS, LOCKE, and GARRETT (**8**), in describing and illustrating the partitioning method of genetic analysis of quantitative characters, give two ways of estimating the theoretical frequency distributions of the genotypes: (*a*) from means and standard errors and (*b*) from the frequency distributions of some of the segregating populations, and then testing against the obtained frequency distributions of other populations. It is evident that, in employing the first method, reliable estimates of the means and standard errors of the genotypes are required. For data in which the genetic and environmental variabilities are following the same scale of measurement and the gene effects cumulate additively on such a scale, the methods given by EMERSON and EAST (**1**) and POWERS (**7**) are adequate. In the event, however, that the environmental and genetic variabilities are not following the same scale or if there are either intra-allelic or interallelic interactions of the genes or both, then some other way of estimating the means of the genotypes must be devised, provided that the partitioning method of gene analysis is to be used.

The purposes of this article are (*a*) to show that such a situation as described above does prevail for number of locules per fruit in the tomato (*Lycopersicon esculentum* Mill.) hybrid Danmark × Johannisfeuer; (*b*) to present and illustrate procedures to be followed that will allow the data to be analyzed by the partitioning method when interactions of genes are involved; (*c*) to present methods and procedures for obtaining additional genetic information from the variances; (*d*) to present a method of determining from the means of the genotypes the effects and interactions of the genes; (*e*) to illustrate the test of reliability; and, finally, (*f*) to interpret the analyzed data.

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Experimental design

The genetic design of the experiment included the Danmark, B_1 to Danmark, F_1, F_2, B_1 to Johannisfeuer, and Johannisfeuer populations. The statistical design of the experiment was a randomized complete block with twenty replications, there being twelve plants of a population grown per plot. In estimating number of locules per fruit, the locules of five fruits per plant were counted in 1939 and only one fruit per plant in 1940. That fewer fruits per plant were used in determining number of locules in 1940 accounts for the greater portion of the increase in size of the variances and standard errors for that year. The symbols used have the same designations as given in previous publications (**5**, **8**).

Scale of measurement

The first procedure is to determine whether a scale of measurement can be

found which is appropriate for describing the biological processes causing the variability.

For the genetic variability this can be done by analyzing the means of the populations. The obtained arithmetic means and the theoretical geometric means for number of locules per fruit and the number of plants classified for populations of Danmark × Johannisfeuer grown in 1939 and in 1940 are given in table 1. If genes are not additive on either the arithmetic or the logarithmic scale, and hence the genetic variability follows neither of these two scales.

That the means of the Danmark, B_1 to Danmark, and F_1 populations do not differ materially in magnitude shows that phenotypic dominance exists in this cross and probably some degree of partial genic dominance. There are at least intra-allelic interactions of genes and likely

TABLE 1

OBTAINED ARITHMETIC MEANS AND THEORETICAL GEOMETRIC MEANS FOR NUMBER OF LOCULES PER FRUIT AND NUMBER OF PLANTS CLASSIFIED FOR POPULATIONS OF DANMARK × JOHANNISFEUER

Population	1939			1940		
	Mean		Plants classified	Mean		Plants classified
	Arithmetic	Geometric		Arithmetic	Geometric	
Danmark	6.19	6.19	228	6.17	6.17	219
B_1 to Danmark	5.89	6.80	231	5.97	6.76	219
F_1	6.07	7.48	209	5.99	7.41	224
F_2	6.84	7.48	215	7.07	7.41	223
B_1 to Johannisfeuer	7.34	8.22	230	7.33	8.13	224
Johannisfeuer	9.03	9.03	224	8.91	8.91	220

the data are following the arithmetic scale, $\bar{F}_1$ should approximate (within the limits attributable to the probable errors of random sampling) $(\bar{P}_1 + \bar{P}_2)/2$, $\bar{B}_1$ to P_1 should approximate $(\bar{F}_1 + \bar{P}_1)/2$, and $\bar{B}_1$ to P_2 should approximate $(\bar{F}_1 + \bar{P}_2)/2$. Such is not the case for either the 1939 or the 1940 data. If the genetic variability is following the logarithmic scale, the $\sqrt{(\bar{P}_1)(\bar{P}_2)}$ should approximate the obtained arithmetic mean of the F_1 population, the $\sqrt{(P_1)(\bar{F}_1)}$ should approximate the obtained mean of the B_1 to Danmark, and $\sqrt{(\bar{F}_1)(\bar{P}_2)}$ should approximate the obtained arithmetic mean of the B_1 to Johannisfeuer. Again such is not the case. Clearly, the effects of the interallelic interactions also. Therefore, it does not seem that any one scale can be found that will describe the genetic variability. Consequently, it is necessary to eliminate genetic variability in making an analysis of the data. This can be accomplished by working with individual genotypes of the populations.

To do this, the scale of measurement followed by the environmental variability needs to be determined. The arithmetic scale was used in taking the data. Therefore, it is logical to ascertain, first, whether the variability in number of locules per fruit is appropriately described by the arithmetic scale. That is, do the data for this character taken on

the arithmetic scale follow the normal probability integral?

The obtained and the theoretical frequency distributions for number of locules per fruit for nonsegregating populations of Danmark × Johannisfeuer grown in 1939 and in 1940 are given in table 2. Also given in this same table calculated by summation of the values for the three nonsegregating populations, as P for testing the fit lies between 0.10 and 0.05. For 1940 the corresponding P values for the Danmark and F_1 populations lie between 0.01 and $1.0 - \infty$, showing that the arithmetic scale is not appropriate for describing the biological

TABLE 2

OBTAINED AND THEORETICAL FREQUENCY DISTRIBUTIONS (NUMERICAL BASIS) FOR NUMBER OF LOCULES PER FRUIT FOR NONSEGREGATING POPULATIONS OF DANMARK × JOHANNISFEUER AND DEGREES OF FREEDOM, CHI SQUARES, AND P VALUES*

YEAR AND POPULATION	UPPER LIMIT OF CLASS (LOCULE NUMBER)												DEGREES OF FREEDOM	CHI SQUARE	P LIES BETWEEN
	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5			
1939:															
Danmark			6	51	90	57	23	1					3	2.845	0.50 and 0.30
Theoretical			8	45	90	66	17	2							
F_1			15	48	77	49	16	4					4	1.014	.95 and 0.90
Theoretical		1	12	47	77	54	16	2							
Johannisfeuer					12	72	70	45	18	6	1		4	12.900	.02 and 0.01
Theoretical				3	17	52	75	54	19	4					
Total			21	99	167	118	111	75	45	18	6	1	7	13.050	.10 and 0.05
Theoretical		1	20	92	170	137	85	79	54	19	4				
1940:															
Danmark		2	32	51	60	33	20	9	6	3	2	1	6	24.420	.01 and $1-\infty$
Theoretical	4	10	23	40	49	43	29	14	5	2					
F_1		8	38	56	50	28	20	15	6	2		1	6	23.098	.01 and $1-\infty$
Theoretical	6	12	27	43	48	43	27	13	4	1					
Johannisfeuer				1	11	37	40	57	35	24	12	3	6	5.825	.50 and 0.30
Theoretical				4	11	28	45	53	42	24	10	3			
Total		10	70	108	121	98	80	81	47	29	14	5	10	41.035	.01 and $1-\infty$
Theoretical	10	22	50	87	108	114	101	80	51	27	10	3			
Total for years:															
Danmark		2	38	102	150	90	43	10	6	3	2	1	6	19.688	.01 and $1-\infty$
Theoretical	4	10	31	85	139	109	46	16	5	2					
F_1		8	53	104	127	77	36	19	6	2		1	6	22.067	.01 and $1-\infty$
Theoretical	6	13	39	90	125	97	43	15	4	1					
Johannisfeuer				1	11	49	112	127	80	42	18	4	6	8.844	.20 and 0.10
Theoretical				4	14	45	97	128	96	43	14	3			
Total		10	91	207	288	216	191	156	92	47	20	6	10	39.855	0.01 and $1-\infty$
Theoretical	10	23	70	179	278	251	186	159	105	46	14	3			

* The values for testing the fit between the obtained and the theoretical frequency distributions are based on the assumption that the environmental variability follows the arithmetic scale.

are the degrees of freedom, chi-square values, and P values for testing the fit between the obtained and the theoretical frequency distributions based on the arithmetic scale. For 1939 the frequency distributions for the Danmark and F_1 populations give an acceptable fit. This is not true, however, of the Johannisfeuer frequency distributions, as P lies between 0.02 and 0.01. This lack of agreement is reflected in the total obtained and in the total theoretical frequency distributions processes causing the environmental variability. However, for 1940 the obtained and the theoretical frequency distributions for the Johannisfeuer population have a P value that lies between 0.50 and 0.30, indicating that for this population the arithmetic scale is appropriate for describing the biological processes causing the environmental variability. As might be expected, the total for years is more discriminatory, and it can be seen that the obtained and the theoreti-

cal frequency distributions for Johannisfeuer are the only two that give an acceptable fit. Even for this acceptable fit the odds against the deviations noted between the two frequency distributions being attributable to chance lie between 4:1 and 9:1. The chi-square and *P* values calculated from the total obtained and the total theoretical frequency distributions cause the arithmetic scale to be rejected as unsuitable for describing the biological processes causing the environmental variability. Also, the total of the chi squares for the nonsegregating populations of 50.599 with 18 degrees of freedom and a *P* value which lies between 0.01 and 1.0 — ∞ lead to rejection of the arithmetic scale.

Logically, the next step is to determine whether the environmental variability follows the logarithmic scale. The obtained and theoretical frequency distributions of number of locules per tomato fruit for the nonsegregating populations of Danmark × Johannisfeuer grown in 1939 and in 1940 are given in table 3. Also in this same table are given the degrees of freedom, chi squares, and *P* values for testing the fit between the obtained and the theoretical frequency distributions based on the assumption that the environmental variability follows the logarithmic scale. For both 1939 and 1940 the obtained and theoretical frequency distributions are a good fit. This is borne out by the fact that none

TABLE 3

OBTAINED AND THEORETICAL FREQUENCY DISTRIBUTIONS (NUMERICAL BASIS) FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) FOR NONSEGREGATING POPULATIONS OF DANMARK × JOHANNISFEUER, AND DEGREES OF FREEDOM, CHI SQUARES, AND *P* VALUES*

YEAR AND POPULATION	UPPER LIMIT OF CLASS IN LOGARITHMS											DEGREES OF FREEDOM	CHI SQUARE	*P* LIES BETWEEN
	0.544068	0.653212	0.740363	0.812913	0.875061	0.929419	0.977724	1.021189	1.060698	1.096910	1.130334	1.161368			
1939:															
Danmark		6	51	90	57	23	1						3	0.617	0.90 and 0.80
Theoretical		5	51	94	57	17	3	1							
F_1		15	48	77	49	16	4						4	2.542	.70 and 0.50
Theoretical		11	55	77	46	15	4	1							
Johannisfeuer					12	72	70	45	18	6	1		4	5.987	.30 and 0.20
Theoretical				1	16	58	77	49	18	4	1				
Total		21	99	167	118	111	75	45	18	6	1		7	8.922	.30 and 0.20
Theoretical		16	106	172	119	90	84	51	18	4	1				
1940:															
Danmark	2	32	51	60	33	20	9	6	3	2	1		5	2.405	.80 and 0.70
Theoretical	6	28	52	52	39	22	11	5	2	1	1				
F_1	8	38	50	50	28	20	15	6	2		1		7	3.781	.90 and 0.80
Theoretical	10	35	53	50	35	20	11	5	3	1	1				
Johannisfeuer			1	11	37	40	57	35	24	12	2	1	6	4.907	.70 and 0.50
Theoretical			2	11	32	51	51	37	20	10	4	2			
Total	10	70	108	121	98	80	81	47	29	14	4	1	9	7.591	.70 and 0.50
Theoretical	16	63	107	113	106	93	73	47	25	12	6	2			
Total for years:															
Danmark	2	38	102	150	90	43	10	6	3	2	1		6	2.473	.90 and 0.80
Theoretical	6	33	103	146	96	39	14	6	2	1	1				
F_1	8	53	104	127	77	36	19	6	2		1		7	3.270	.90 and 0.80
Theoretical	10	46	108	127	81	35	15	6	3	1	1				
Johannisfeuer			1	11	49	112	127	80	42	18	3	1	6	1.284	.98 and 0.95
Theoretical			2	12	48	109	128	86	38	14	5	2			
Total	10	91	207	288	216	191	156	92	47	20	5	1	9	5.769	0.80 and 0.70
Theoretical	16	79	213	285	225	183	157	98	43	16	7	2			

* The values for testing the fit between the obtained frequency distributions and the theoretical frequency distributions are based on the assumption that the environmental variability follows the logarithmic scale.

of the chi-square values for the individual populations is larger or smaller than would be expected on the basis of random sampling. Also the chi-square values for the total frequency distribution resulting from adding those of the nonsegregating populations for any given year and resulting from calculations after adding the total frequency distributions for the two years (1939 and 1940) show that the differences between the obtained and the theoretical frequency distributions are no greater than can be explained by chance. These chi-square values show not only that the individual population frequency distributions give a good fit to the theoretical values based on a transformation of the individual plant data to logarithms but that the totals within years and for years do also, and, therefore, there is no consistent skewness of the data as a whole.

The total degrees of freedom, chi squares, and *P* values of these totals for the three nonsegregating populations for 1939 and 1940 individually and for the two years summed are, respectively, 11 and 9.146, and *P* lies between 0.70 and 0.50; 18 and 11.093, and *P* lies between 0.90 and 0.80; and 19 and 7.027, and *P* lies between 1 and 0.99. The total of the chi squares for the three nonsegregating generations for any given year or the years taken collectively is not larger than expected on the basis of chance. In fact, the chi-square value resulting from summing those for the two years shows that deviations between the obtained and the theoretical frequency distributions are not so great as would be expected by chance. However, *P* is just slightly larger than 0.99.

Since the Danmark, F_1, and Johannisfeuer populations represent three different genotypes and since the environmental variabilities of all follow the normal probability integral on the logarithmic scale, it seems probable that the environmental variabilities of all the genotypes of the segregating populations are following the normal probability integral on the logarithmic scale. However, even though unlikely, the environmental variabilities of some genotypes could be following another scale. Evidence that such is not the case will be presented later when considering the obtained and the theoretical genetic variances. The original individual plant data were transformed to logarithms before making the analyses which follow.

Provisional genetic hypothesis

In gene analyses, provisional genetic hypotheses are postulated as working models, and then their validity is tested. Usually, it is easiest to set up a logical provisional hypothesis from an examination of the end-classes of the frequency distributions expressed in percentages. For number of locules per fruit, frequency distributions expressed in percentages for the different populations of the cross Danmark × Johannisfeuer grown in 1939 are given in table 4. The frequency distribution for the B_1 to Danmark, at both extremes, exceeds those of the Danmark and F_1 populations. The same is true for the frequency distribution of the F_2 population. Without detailed study, the percentage of individuals falling beyond 1.021189 does not appear to differ materially for the F_2, B_1 to Johannisfeuer, and Johannisfeuer populations. Each of the three segregating populations has a larger percentage of individuals in the 0.653212 and lower classes than does any of the nonsegregating populations. The results noted above are those expected if the parents are differentiated by few pairs of genes and if both parents carry some

genes which are at least partially dominant for fewer locules per fruit.

The simplest possible genetic hypothesis complying with the immediately preceding observations is that the parents of the Danmark × Johannisfeuer cross are differentiated by two pairs of genes. In testing this hypothesis, these two pairs of genes will be designated as *AAbb* in Danmark, as *AaBb* in F_1, and as *aaBB* in Johannisfeuer. Then the theoretical genotypes of the segregating populations are as listed in table 5.

TABLE 4

OBTAINED FREQUENCY DISTRIBUTIONS (PERCENTAGE BASIS) FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) FOR DIFFERENT POPULATIONS OF THE CROSS DANMARK × JOHANNISFEUER GROWN IN 1939, AND THEORETICAL FREQUENCY DISTRIBUTIONS OF NONSEGREGATING POPULATIONS

POPULATION	UPPER LIMIT OF CLASS IN LOGARITHMS											
	0.544068	0.653212	0.740363	0.812913	0.875061	0.929419	0.977724	1.021189	1.060698	1.096910	1.130334	1.161368
Danmark		2.6	22.4	39.5	25.0	10.1	0.4					
Theoretical		2.3	22.2	41.0	25.2	7.6	1.5	0.2.				
B_1 to Danmark	0.9	13.0	29.9	26.0	19.0	7.8	2.1	0.9	0.4			
F_1		7.2	23.0	36.8	23.4	7.7	1.9					
Theoretical	0.1	5.1	26.4	36.8	22.1	7.4	1.7	0.3	0.1			
F_2	1.9	12.6	16.7	24.7	12.1	8.4	7.4	6.0	6.5	2.3	1.4	
B_1 to Johannisfeuer		13.9	19.6	12.6	5.2	8.3	15.2	14.3	8.3	1.3	1.3	
Johannisfeuer					5.4	32.1	31.2	20.1	8.0	2.7	0.5	
Theoretical				0.5	7.4	25.8	34.4	21.7	7.9	1.9	0.3	0.1

Estimation of the theoretical means of the genotypes

If that part of the partitioning method in which the theoretical frequency distributions are estimated from the means and standard errors is to be employed in analyzing the data, some method of estimating the theoretical means of the genotypes must be devised. The question arises as to whether the means of the different populations listed in table 6 can be used in estimating the theoretical means of the genotypes of the segregating populations by methods given by POWERS *et al.* (**7**, **8**). These methods are suitable when there are no interactions of the genes or when the nature of the interactions is simple and can be readily determined. The frequency distributions listed in table 4, together with the means given in table 6, provide some information as to the simplicity of the nature of the interactions of the genes. From an examination of the means of the Danmark, B_1 to Danmark, and F_1 populations one might conclude that genic dominance is complete. If such is the case, the frequency distribution (table 4) of the F_1 population should have a higher percentage of its plants in classes less than 0.740363 than do the frequency distributions of the B_1 to Danmark, F_2,

TABLE 5

ESTIMATED MEANS FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) OF GENOTYPES OF SEGREGATING POPULATIONS OF DANMARK × JOHANNISFEUER GROWN IN 1939

B_1 to Danmark*		F_2			B_1 to Johannisfeuer*	
Genotype	Mean	Genotype	Mean	Theoretical percentage	Genotype	Mean
AABb	0.657900	*AABB*	0.598320	6.25	*AaBB*	0.652458
AAbb	.786440†	*AABb*	0.657900	12.50	*AaBb*	.776403†
AaBb	.776403†	*AAbb*	0.786440†	6.25	*aaBB*	.952126†
Aabb	.831417	*AaBB*	0.652458	12.50	*aaBb*	.997198
		AaBb	0.776403†	25.00		
Theoretical	.763040	*Aabb*	0.831417	12.50	Theoretical	.844546
Obtained	0.759480	*aaBB*	0.952126†	6.25	Obtained	0.840356
		aaBb	0.997198	12.50		
		aabb	1.035071	6.25		
		Theoretical	0.797220			
		Obtained	0.811669			

* Each genotype comprises 25% of the backcross populations.

† These are the obtained means of Danmark, F_1, and Johannisfeuer, respectively.

TABLE 6

MEANS AND THEIR STANDARD ERRORS AND VARIANCES AND THEIR STANDARD ERRORS FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) FOR TOMATO POPULATIONS

Constant and population	1939	1940
Means and standard errors:		
Danmark	0.786440 ± 0.004806	0.773933 ± 0.008017
B_1 to Danmark	.759480 ± .007080	.748490 ± .007289
F_1	.776403 ± .007120	.758839 ± .009573
F_2	.811669 ± .008314	.811895 ± .013288
B_1 to Johannisfeuer	.840356 ± .008258	.828537 ± .012576
Johannisfeuer	.952126 ± .004065	.942547 ± .005204
Variances and standard errors:		
Danmark	.004486 ± .000407	.013879 ± .001804
B_1 to Danmark	.009397 ± .000724	.022921 ± .001902
F_1	.005683 ± .000589	.016234 ± .001110
F_2	.019035 ± .002286	.033026 ± .002775
B_1 to Johannisfeuer	.022845 ± .001332	.033435 ± .003007
Johannisfeuer	0.002973 ± 0.000366	0.006631 ± 0.000564

and B_1 to Johannisfeuer populations. The opposite is true in all three comparisons. Also, the B_1 to Danmark population has a higher percentage of plants falling beyond the 0.929419 class than does either the F_1 or the Danmark populations. Finally, as previously pointed out, the percentages (10.2, 10.9, and 11.2) of plants falling beyond the 1.021189 class for the F_2, B_1 to Johannisfeuer, and Johannisfeuer populations, respectively, are not materially different. The behavior of these means and frequency distributions cannot be explained by genetic models, assuming equal effects of genes and that the nature of the interactions of the genes is simple. Clearly, the methods of estimating the means of the genotypes previously given by POWERS *et al.* (**7**, **8**) are not suited to the genetic analyses of these data. The method developed and illustrated in this article utilizes the obtained frequency distributions in estimating the theoretical means of the genotypes. It requires an estimation of the standard errors of a single determination for the theoretical genotypes. Since the standard errors, in turn, are calculated from the variances, estimation of the standard errors resolves into estimating the variances of the theoretical genotypes.

The variances and their standard errors for the different populations are given in the second part of table 6. In both years the variances for the Danmark and F_1 populations are larger than the variances for the Johannisfeuer populations. The differences are statistically significant. From an examination of the means of the logarithms and their corresponding variances, it can be seen that the larger means do not have the larger variances. In fact, the mean of Johannisfeuer is significantly larger than that of either Danmark or the F_1, but the variance of Johannisfeuer is smaller. It is apparent that, even though the environmental variability is following the logarithmic scale, a transformation to logarithms does not equalize the variances. For this reason, in estimating the means of the genotypes from the obtained frequency distributions, the variance of the population which the genotype more nearly approaches in genetic constitution is used. For example, to estimate the mean and the frequency distribution of the *AABb* genotype, the standard error of a single determination derived from the variance of Danmark is employed, and for the same calculations for the *aaBb* genotype the variance of Johannisfeuer is used. Whether this procedure is justified can be tested, since the fit should be good between the obtained and the theoretical genetic variances if such is the case. The evidence will be presented under a later section dealing with the obtained and the theoretical genetic variances.

B_1 TO DANMARK POPULATION.—The genotypes of the B_1 to Danmark population are listed in table 5. The mean of the Danmark population furnishes the best available estimate of the mean of the *AAbb* genotype, and the mean of the F_1 population the best estimate of the mean of the *AaBb* genotype. Only the means for the *AABb* and the *Aabb* genotypes remained to be determined. First, the mean of the *Aabb* genotype will be estimated. From table 4 it can be seen that, on the basis of the normal probability integral (theoretical), only 1.7% of the plants of the *AAbb* (Danmark) genotype would be expected to fall beyond the 0.929419 class. Likewise, the same table shows that 2.1% of the plants of the *AaBb* genotype (F_1) would be expected to fall beyond this same class. Since a smaller number of locules is at least

partially dominant, there would be fewer plants of the *AABb* genotype falling beyond the 0.929419 class than of the *AAbb* genotype. It will be shown later that no plants of the *AABb* genotype would be expected to fall beyond this class. Since plants of each of the four genotypes (table 5) comprise 25% of the B_1 to Danmark population, the expected proportion of plants of the *AAbb* and *AaBb* genotypes falling beyond the 0.929419 class is 0.25(1.7 + 2.1), or 1.0%. This latter value subtracted from 3.4% (the total percentage of the B_1 to Danmark plants falling beyond this class) leaves 2.4% attributable to plants of the *Aabb* genotype. To estimate the mean of this genotype, it is necessary to know the proportion of the plants that would be expected to fall beyond 0.929419 if all the population had been made up of *Aabb* genotypic plants instead of only 25% being of this genotype. The proportion 2.4/0.25 gives a value of 9.6%. This value and PEARSON's (**4**) tables are used to estimate the mean of the *Aabb* genotype. The value of 9.6% subtracted from 100% gives a value of 90.4%, which in PEARSON's tables has an x value of 1.30.

Letting y equal the mean value sought, s_d the standard deviation of a single determination, and z the upper limit of the class under consideration, the formula for y is $z - s_d x$ if z is greater than y or $z + s_d x$ if z is less than y. The standard error of the F_1 population, as calculated from the variance of this generation given in table 6, is 0.075386, and it furnishes a satisfactory estimate of s_d for the *Aabb* genotype. The upper limit of the class under consideration is 0.929419. Since 90.4% of the *Aabb* genotypic plants had values less than this upper limit, z must be greater than y, and the proper formula is $y = z - s_d x$. Then, by substituting these values in the above formula and solving the equation, the mean of *Aabb* is found to be 0.929419 − (0.075386)(1.30), or 0.831417.

The mean of plants of the *AABb* genotype is obtained in a similar manner. From an examination of table 5 it is apparent that, of the genotypes of the B_1 to Danmark population, plants of the *AABb* genotype would have the smallest mean value. A study of table 4 reveals that 13.9% of the B_1 to Danmark population fell in the class having an upper limit of 0.653212 or fell in lower classes. This same table shows that 2.3% of the plants of the *AAbb* (Danmark) genotype and 5.2% of the plants of the *AaBb* (F_1) genotype would be expected to fall in these same classes. Using the standard error of the F_1 (0.075386, the square root of the variance for a single determination, table 6) and the estimated mean of 0.831417, the percentage of plants of the *Aabb* genotype expected to fall in the class having an upper limit of 0.653212 or in lower classes can be calculated in the usual manner (**8**) and is 0.9. Since each of the four genotypes listed under the B_1 to Danmark population of table 5 comprises 25% of the population, the amount of the 13.9% accounted for by the *AAbb*, *AaBb*, and *Aabb* genotypes is 0.25 (2.3 + 5.2 + 0.9), or 2.1%. Then plants of the *AABb* genotype account for 11.8% (13.9 − 2.1) of the 13.9% of the B_1 to Danmark population falling in the class having an upper limit of 0.653212 or in lower classes. But, since this genotype comprises only 25% of the B_1 to Danmark population, in a population having all the plants of the *AABb* genotype the theoretical percentage of the plants expected to fall in these classes is 11.8/0.25, or 47.2%. This value is used to estimate the mean of the *AABb* geno-

typic plants. By substituting in the formula given previously, $y = 0.653212 + (0.066978)(0.07)$, or 0.657900. The value 0.066978 is obtained from the variance of Danmark (table 6). The latter part of the equation must be added to 0.653212 (z) because 47.2% is less than 50.0%, and hence the mean of the *AABb* plants must be greater than 0.653212. This is the method of determining whether the value of $s_d x$ should be added to or subtracted from the value of z to give y (the estimated mean).

By the use of PEARSON's tables (4) and the standard deviation of 0.066978, it can be shown that plants of the genotype *AABb* with a mean of 0.657900 would not be expected to fall in classes having an upper limit greater than 0.929419. Consequently, the estimate of the mean of plants of the *Aabb* genotype was justified as based on the supposition that plants of the *AABb* genotype did not fall in classes having an upper limit greater than 0.929419.

B_1 TO JOHANNISFEUER POPULATION.—In a consideration of the genotypes of the B_1 to Johannisfeuer population (table 5), the best estimates of the means of the *AaBb* and *aaBB* genotypes are the means of the F_1 and Johannisfeuer populations, respectively. This leaves only the means of the *aaBb* and *AaBB* genotypes to be estimated. From table 4 it can be seen that only 0.1% of the plants of the *AaBb* (F_1) genotype and 10.2% of plants of the *aaBB* (Johannisfeuer) genotype would be expected to fall in classes having an upper limit greater than 1.021189. Hence 0.25 (0.1 + 10.2), or 2.6% of the plants of the B_1 to Johannisfeuer population falling in classes having an upper limit greater than 1.021189, would be expected to be of the *AaBb* and *aaBB* genotypes. Since plants of the *AaBB* genotype would be expected to have a mean value smaller than that of the F_1 (*AaBb* genotype), no plants of the *AaBB* genotype would be expected to fall in the classes having an upper limit greater than 1.021189. Then, of the 10.9% of plants of the B_1 to Johannisfeuer population falling in these higher classes, 8.3% (10.9 − 2.6) would be of the *aaBb* genotype. In a population made up entirely of *aaBb* plants, 33.2% (8.3/0.25) would be expected to fall in the classes having an upper limit greater than 1.021189. This percentage value, together with the standard error (0.054525) of the Johannisfeuer population (square root of the variance, table 6), and the upper class limit under consideration are used to estimate the mean of the *aaBb* plants. By substituting these values in the formula $y = z - s_d x$ and solving the equation, the mean value of plants of the *aaBb* genotype is found to be 0.997198. In obtaining this value, a value of x equal to 0.44 was used instead of the equally appropriate value of 0.43.

Only the mean of plants of the *AaBB* genotype remains to be estimated for the B_1 to Johannisfeuer population. From an examination of table 4 it can be seen that 13.9% of the plants of the B_1 to Johannisfeuer population fell in the class having an upper limit of 0.653212 and that no plants fell in lower classes. The theoretical frequency distributions of the same table show that only plants of the *AaBb* genotype would be expected to have plants falling in this class, as the lowest class in which plants of the Johannisfeuer genotype were expected to fall was the 0.812913 class. The percentage of plants of the *AaBb* genotype expected in the class having an upper limit of 0.653212 or in lower classes is (5.2)(0.25), or 1.3%. Therefore, 12.6% (13.9 − 1.3) must be of the *AaBB* genotype. Consequently, in a population in

which all the plants are of the *AaBB* genotype, 50.4% (12.6/0.25) would be expected to fall in the class having an upper limit of 0.653212 or in lower classes. This percentage value is used in estimating the mean of the *AaBB* genotype by the use of the formula given previously. The best available estimate of s_d is that of the F_1 (square root of the variance, table 6) and is 0.075386. By substitution of the proper values in the formula and solving the equation, the mean of the *AaBB* genotype is estimated to be 0.652458.

F_2 POPULATION.—The only genotypes of the F_2 population whose means are unknown (table 5) are *AABB* and *aabb*.

First, an estimate of the mean for plants of the *AABB* genotype will be obtained. An examination of table 4 reveals that the only populations having plants falling in the 0.544068 class are the F_2 and B_1 to Danmark. It can be seen from table 4 that none of the plants of the *AAbb* (Danmark) genotype would be expected to fall in the class having an upper limit of 0.544068. Hence, other than plants of the *AABB* genotype, the only plants able to account for the percentage of plants noted for this class are those of the *AABb* genotype. Theoretically, plants of the *AABb* genotype comprise 12.5% (table 5) of the F_2 population. Previously, the mean of this genotype has been estimated as 0.657900, and the best estimate of the standard error is that of Danmark (square root of the variance, table 6) and is 0.066978. From these values the theoretical percentage of plants of the *AABb* genotype falling in class 0.544068 is 4.5% for populations made up entirely of plants of this genotype. Since plants of the *AABb* genotype theoretically comprise only 12.5% (table 5) of the F_2 population, these plants account for (4.5) (12.5)/100, or 0.6% of the 1.9% falling in the class having an upper limit of 0.544068. The balance of 1.3% is attributable to plants of the *AABB* genotype. Theoretically, plants of the *AABB* genotype comprise only 6.25% of the F_2 population. Consequently, when all the population is made up of plants of the *AABB* genotype, the percentage of the population expected to fall in the class having an upper limit of 0.544068 is 1.3/0.0625, or 20.8%. This value is used in estimating the mean of plants having the *AABB* genotype. By substituting the proper values in the usual formula, the mean of the *AABB* genotype equals 0.544068+ (0.066978) (0.81), or 0.598320.

The mean of the *aabb* genotype remains to be estimated. This can be done from the percentage of plants of the F_2 population (table 4) falling in classes having an upper limit greater than 1.060698. A study of table 5 reveals that some plants of the *Aabb* genotype having a mean of 0.831417 and a standard error of 0.075386 might fall in classes having greater values than 1.060698. By using PEARSON's tables (4) and the methods previously given, it can be determined that only 0.1% of a population of plants of this genotype would be expected to fall in these classes. Therefore, as this genotype comprises only 12.5% of the F_2 population, it is evident that it need not be considered further in the calculation of the mean of plants having the *aabb* genotype. The two genotypes that need to be considered are *aaBB* and *aaBb*, which have means of 0.952126 and 0.997198, respectively. An examination of table 4 shows that the percentage of the population of plants of the first genotype expected to fall in classes having a larger value than 1.060698 is 2.3 (Johannisfeuer theoretical). By using PEARSON's tables (4), the value calculated for

the second genotype is 12.3%. Theoretically, plants of the *aaBB* and *aaBb* genotypes comprise 6.25 and 12.5% of the F_2 population, respectively. Consequently, these two genotypes account for (2.3) (6.25) + (12.3) (12.5), or 1.7% of the total of 3.7% of F_2 plants falling in classes having an upper limit greater than 1.060698. The balance of 2.0% is attributable to the *aabb* genotype. This genotype theoretically comprises 6.25% of the F_2 population, so that a population made up entirely of plants of the *aabb* genotype would be expected to have 32.0% (2.0/0.0625) of its plants falling in classes having an upper limit greater than 1.060698. By substituting the proper values in the formula $y = z - s_d x$ and solving the equation, the estimated mean of plants of the *aabb* genotype is 1.060698 − (0.054525) (0.47), or 1.035071.

TABLE 7

MEANS, STANDARD ERRORS OF A SINGLE DETERMINATION, AND THEORETICAL PERCENTAGE FREQUENCY DISTRIBUTIONS FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) FOR GENOTYPES OF F_2 AND BACKCROSS POPULATIONS, DANMARK × JOHANNISFEUER CROSS GROWN IN 1939

GENOTYPE AND POPULATION	MEAN	STANDARD ERROR	UPPER LIMIT OF CLASS IN LOGARITHMS																PERCENTAGE OF F_2 POPULATION
			0.397940	0.544068	0.653212	0.740363	0.812913	0.875061	0.929419	0.977724	1.021189	1.060698	1.096910	1.130334	1.161368	1.190332	1.217484		
AABB	0.598320	0.066978	0.1	20.8	58.5	18.9	1.6	0.1											6.25
*AaBB**	0.652458	.075386		7.5	42.9	37.5	10.4	1.5	0.2										12.50
AABb†	0.657900	.066978		4.5	42.7	41.9	9.9	0.9	0.1										12.50
AAbb†	0.786440	.066978			2.3	22.2	41.0	25.2	7.6	1.5	0.2								6.25
AaBb‡	0.776403	.075386	0.1	5.1	26.4	36.8	22.1	7.4	1.7	0.3	0.1								25.00
Aabb†	0.831417	.075386		0.9	10.4	28.8	31.8	18.4	7.1	2.0	0.5	0.1							12.50
*aaBB**	0.952126	.054525				0.5	7.4	25.8	34.4	21.7	7.9	1.9	0.3	0.1					6.25
*aaBb**	0.997198	.054525					1.3	9.4	25.2	31.1	20.7	8.9	2.7	0.6	0.1				12.50
aabb	1.035071	0.054525						0.2	2.4	12.1	25.4	28.0	19.0	8.9	3.0	0.8	0.2		6.25
B_1 to Danmark			1	1	12.8	25.2	29.1	20.0	8.4	2.6	0.6	0.2							
F_2			2.8	15.9	20.4	18.0	12.0	7.6	7.5	7.2	4.9	2.4	0.9	0.3	0.1				
B_1 to Johannisfeuer			1.9	12.0	16.0	11.9	8.1	10.7	15.3	13.3	7.2	2.7	0.7	0.2					

* Genotypes occurring in the backcross to Johannisfeuer population.

† Genotypes occurring in the backcross to Danmark population.

‡ Genotypes occurring in both backcross populations.

Obtained and theoretical frequency distributions

The theoretical means of the genotypes listed in table 5, together with the variances of a single determination listed in table 6, allow the calculation of the theoretical frequency distributions. The standard errors for a single determination are obtained by taking the square root of the variances listed in table 6. The variance of Danmark was used in calculating the standard error of a single determination of the *AABB*, *AABb*, and *AAbb* genotypes; that of the F_1 for the *AaBB*, *AaBb*, and *Aabb* genotypes; and that of Johannisfeuer for the *aaBB*, *aaBb*, and *aabb* genotypes. With these means and standard errors of a single determination available, the theoretical percentage frequency distributions for each of the genotypes were calculated by methods given by POWERS *et al.* (8). They are listed in table 7. From these theoretical percentage frequency distributions of the different genotypes were calculated the theoretical percentage frequency distributions of the B_1 to Danmark, F_2, and B_1 to Johannisfeuer populations given at the bottom of this same table. The method of calculation is that

given by POWERS *et al.* (**8**). These percentage frequency distributions, together with the number of plants per population given in table 1, were used in calculating the theoretical frequency distributions (expressed in numbers) for 1939 given in table 8.

The theoretical means of the genotypes for 1940 were calculated from the theoretical means of the genotypes for 1939 of table 7 and the means of the nonsegregating populations for 1940. The means of the nonsegregating populations for both years were totaled, and the total for 1940 was expressed as a percentage of 1939. Thus $(0.773933 + 0.758839 + 0.942547)/(0.786440 + 0.776403 + 0.952126)$ gives 98.423440%. The means of the genotypes other than *AAbb*, *AaBb*, and *aaBB* were estimated by multiplying their respective means for 1939 listed in table 7 by 98.423440 and dividing by 100.0. Again, the best estimates of the means for the *AAbb*, *AaBb*, and *aaBB* genotypes are the 1940 obtained means of the Danmark, F_1, and Johannisfeuer populations, respectively. The standard errors were calculated by

TABLE 8

OBTAINED AND THEORETICAL FREQUENCY DISTRIBUTIONS (NUMERICAL BASIS) FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS), AND DEGREES OF FREEDOM, CHI SQUARES, AND *P* VALUES FOR TESTING THE FIT BETWEEN OBTAINED AND THEORETICAL DATA FROM DANMARK × JOHANNISFEUER CROSS

YEAR AND POPULATION	UPPER LIMIT OF CLASS IN LOGARITHMS												DEGREES OF FREEDOM	CHI SQUARE*	*P* LIES BETWEEN
	0.544068	0.653212	0.740363	0.812913	0.875061	0.929419	0.977724	1.021189	1.060698	1.096910	1.130334	1.161368			
1939:															
B_1 to P_1†	2	30	69	60	44	18	5	2	1				2	1.416	0.50 and 0.30
Theoretical	3	30	58	67	46	19	6	2							
F_2	4	27	36	53	26	18	16	13	14	5	3		5	4.550	.50 and 0.30
Theoretical	6	34	44	39	26	16	16	15	11	5	2	1			
B_1 to P_2†		32	45	29	12	19	35	33	19	3	3		5	3.332	.70 and 0.50
Theoretical	4	28	37	27	19	25	35	31	16	6	2				
Total	6	89	150	142	82	55	56	48	34	8	6		6	2.844	.90 and 0.80
Theoretical	13	92	139	133	91	60	57	48	27	11	4	1			
1940:															
B_1 to P_1†	13	53	45	42	18	22	10	3	4	5	3	1	7	7.990	.50 and 0.30
Theoretical	15	38	50	44	32	19	10	5	3	1	1	1			
F_2	17	27	39	28	34	16	17	9	11	14	5	6	9	10.216	.50 and 0.30
Theoretical	19	36	38	31	25	21	18	14	10	6	3	2			
B_1 to P_2†	19	21	29	34	26	20	19	20	19	7	4	6	9	7.340	.70 and 0.50
Theoretical	15	26	27	24	25	29	28	22	14	7	4	3			
Total	49	101	113	104	78	58	46	32	34	26	12	13	10	10.794	0.50 and 0.30
Theoretical	49	100	115	99	82	69	56	41	27	14	8	6			

* Totals for the segregating populations for 1939: degrees of freedom, 12; chi square, 9.298; and *P* lies between 0.70 and 0.50. For 1940: degrees of freedom, 25; chi square, 25.546; and *P* lies between 0.50 and 0.30. And for the two years, the $\sqrt{2\chi^2} - \sqrt{2n-1}$ is −0.196, and *P* is 0.84.

† P_1 is Danmark and P_2 is Johannisfeuer.

taking the square root of the variances of the Danmark, F_1, and Johannisfeuer populations listed under the 1940 column heading of table 6. The variance corresponding to the one employed in 1939 for estimating the standard errors of a single determination was used. For example, the 1940 variance of Danmark was used to estimate the standard errors of a single determination for the *AABB*, *AABb*, and *AAbb* genotypes. With these means and standard errors available, the theoretical frequency distributions listed in table 8 for 1940 were calculated in the same manner as those for 1939.

The obtained frequency distributions were listed in table 8 also, and the chi-square values were calculated for testing whether the deviations between the obtained and the theoretical frequency distributions were greater than could be explained by the probable errors of random sampling. As shown in table 8, the data were grouped in calculating chi-square values so that the theoretical frequency distribution had at least ten individuals in each class. As shown by the grouping in table 4, in each segregating population two degrees of freedom (end-classes) were employed in estimating the means of certain genotypes for 1939. As a consequence, the two degrees of freedom used in estimating the means are deducted from the total to give those listed in table 8 for 1939. This was not true for the 1940 data. Since the means of the different genotypes were estimated from the 1939 data and from the data for the nonsegregating populations of 1940, all the degrees of freedom are available for calculating the chi-square values.

For the data given in table 8, in no case is the value of P less than 0.30 or greater than 0.90. The total of the chi-square values for 1939 has a P value lying between 0.70 and 0.50; for 1940 a P value lying between 0.50 and 0.30; and for the two years the $\sqrt{2\chi^2} - \sqrt{2n-1}$ is -0.196 and the value of P is 0.84 (**2**). These values show that the deviations between the obtained and the theoretical frequency distributions are those expected on the basis of random sampling. Moreover, the theoretical means for the segregating populations for 1939 are in close agreement with the obtained means (bottom of table 5). Thus this comparison of the means provides additional evidence which supports the contention postulating that the parents are differentiated by two pairs of genes and that the theoretical means of the genotypes given in table 5 are reliable estimates.

The close agreement between the obtained and the theoretical frequency distributions is rather conclusive in showing that two pairs of genes are differentiating the parents as regards number of locules per fruit. Also it follows that the theoretical mean values of the different genotypes are reliable estimates of the actual mean values of these genotypes.

In comparing these means to determine the nature of the interactions of the genes, as is done later, their standard errors are needed. It is not possible to calculate the standard errors of these means in the usual manner. However, a reliable estimate of their standard errors can be obtained from the variances of the nonsegregating generations. This must be so because the means of the genotypes were estimated by the use of these variances. Also, that such must be the case is shown from theoretical considerations. In any segregating population the genotypes would be expected to occur at random in the field. This has been shown to be the case for other characters in these experiments (**5**). It follows that the total variances for the non-

segregating generations given in table 6 provide reliable estimates of the variances of plants of these genotypes. Such being the case, the theoretical number of individuals and the estimates of variance furnish a reliable basis for estimating the standard errors of the theoretical means of the genotypes. The means and standard errors of the genotypes, the populations from which the means were estimated, and the theoretical number of plants of the designated genotype in the population from which the means were estimated are given in table 9.

TABLE 9

MEANS AND STANDARD ERRORS FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) OF GENOTYPES, THE POPULATIONS FROM WHICH MEANS WERE ESTIMATED, AND THEORETICAL NUMBER OF PLANTS OF DESIGNATED GENOTYPE IN POPULATION FROM WHICH MEANS WERE ESTIMATED, FOR DANMARK × JOHANNISFEUER GROWN IN 1939

Genotype	Mean	Population	No. of plants
BB:			
AA	0.598320±0.018576	F_2	13
Aa	0.652458± .009899	B_1 to P_2	58
aa	0.952126± .004065	P_2	224
Bb:			
AA	0.657900± .008794	B_1 to P_1	58
Aa	0.770403± .007120	F_1	209
aa	0.997198± .007159	B_1 to P_2	58
bb:			
AA	0.786440± .004806	P_1	228
Aa	0.831417± .009899	B_1 to P_1	58
aa	1.035071±0.015122	F_2	13

Since the best available estimates of the means of the *AAbb*, *AaBb*, and *aaBB* genotypes are the obtained means of the Danmark, F_1, and Johannisfeuer populations, respectively, it follows that the standard errors of the means of the same populations are the best available estimates of the standard errors of the means of these genotypes. The standard errors of the means of the *AABB* and *AABb* genotypes are estimated from the variance of the Danmark population and are $\sqrt{0.00448564/13}$, or 0.018576, and $\sqrt{0.00448564/58}$, or 0.008794. The value 13 is 6.25% of 215, the number of individual plants in the F_2 population (see table 1), and the value 58 is 25% of 231, the number of individual plants in the B_1 to Danmark population. The theoretical numbers of plants for the other genotypes were calculated in a similar manner. The standard errors of the means of the *Aabb* and *AaBB* genotypes are calculated from the variance of the F_1 population and are $\sqrt{0.00568296/58}$, or 0.009899 for both, as the *n* value for these two genotypes is the same. The standard errors of the *aaBb* and *aabb* genotypes are calculated from the variance of the Johannisfeuer population and are $\sqrt{0.00297286/58}$, or 0.007159, and $\sqrt{0.00297286/13}$, or 0.015122.

Obtained and theoretical genetic variances

The chi-square values previously discussed show that the data for the frequency distributions for 1939 and 1940 are in accord with the hypothesis that Danmark and Johannisfeuer are differentiated by two pairs of genes as regards number of locules per fruit. Such being the case, the means of the genotypes listed in table 7 can be used to estimate the genetic variances. It follows that these theoretical genetic variances should not differ significantly from the genetic variances obtained by subtracting the environmental variances of the segregating populations from the total variances of these populations given in table 6. The environmental variances of the segregating populations were estimated

from those of the nonsegregating populations as follows.

To obtain an estimate of the environmental variance of the B_1 to Danmark population, the environmental variances of Danmark and of the F_1 given in table 6 were averaged. This value was subtracted from the total variance of the B_1 to Danmark. The procedure was as follows: $0.009397 - [(0.004486 + 0.005683)/2]$, which gives 0.004313, the obtained variance of the B_1 to Danmark for 1939. To estimate the environmental variance of the F_2, the average of the environmental variances of the three nonsegregating populations was used, and for the B_1 to Johannisfeuer, the average of the environmental variances of the F_1 and Johannisfeuer populations. Subtraction of these estimated environmental variances from the total variances of the respective populations (table 6) gave the obtained genetic variances listed in table 10. The corresponding values for 1940 were calculated similarly, except that the data for that year were used. The theoretical genetic variances were calculated from the theoretical means of the genotypes of table 5 by standard methods. In making these calculations for the F_2 generation, it must be taken into account that all genotypes do not occur with equal frequency.

Measurements of the reliability of these variances are needed. The standard errors are used. The standard error of the variance for any given population of table 6 is calculated from variances for the ten blocks (replications) of that population by use of standard formulas. Since the obtained genetic variances represent a difference between the total variances obtained and the estimated environmental variances, their standard errors are calculated from the formulas for obtaining standard errors of difference. The procedure for the F_2 population is illustrated as follows:

$\sqrt{(0.000407^2 + 0.000589^2 + 0.000366^2)/9}$ equals 0.000268, and

$$\sqrt{0.000268^2 + 0.002286^2}$$

equals 0.002302, the standard error for the F_2 given in table 10 for 1939. The data for calculating the standard errors were taken from table 6.

The standard errors for the theoretical genetic variances were obtained from the nonsegregating population variances (table 6) as follows:

$$\sqrt{(0.000407^2 + 0.000589^2)/2}$$

For the B_1 to Danmark it is 0.000506. In other words, the average of the variances of the variances for the Danmark

TABLE 10

OBTAINED GENETIC VARIANCES AND THEIR STANDARD ERRORS AND THEORETICAL GENETIC VARIANCES AND THEIR STANDARD ERRORS FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) FOR SEGREGATING POPULATIONS OF DANMARK × JOHANNISFEUER

POPULATION	GENETIC VARIANCE		
	1939 obtained	Theoretical	1940 obtained
B_1 to Danmark	0.004313 ± 0.000807	0.004114 ± 0.000506	0.007865 ± 0.002177
F_2	.015554 ± .002302	.017815 ± .000464	.020778 ± .002870
B_1 to Johannisfeuer	0.018517 ± 0.001376	0.019104 ± 0.000490	0.022003 ± 0.003071

and F_1 populations gives a satisfactory estimate of the variance of the theoretical genetic variance for the B_1 to Danmark population. It follows that the variances of the variances for the three nonsegregating populations give a satisfactory estimate of the variance of the theoretical genetic variance for the F_2 population. The variances of the variances for the F_1 and Johannisfeuer provide a satisfactory estimate of the variance of the theoretical genetic variance of the B_1 to Johannisfeuer population.

From an examination of the data given in table 10 it can be determined that in no case are the theoretical genetic variances significantly different from those obtained for either 1939 or 1940, if odds of 19:1 are accepted as a criterion of statistical significance. An average of the two years would give a still better fit between the obtained and the theoretical genetic variances.

This is convincing evidence in support of the genetic hypothesis advanced to explain the inheritance of number of locules per fruit. Also, the close agreement between the obtained and the theoretical genetic variances furnishes rather convincing evidence that the environmental variabilities of all the genotypes of table 9 follow the logarithmic scale and therefore supply the final evidence needed to justify the transformation of the original individual plant data to logarithms. In other words, regardless of genotype, the environmental variabilities follow the normal probability integral when the data are transformed to logarithms. Also, this close agreement between obtained and theoretical genetic variances shows that it was sound to use the methods of estimating the variances (table 7) of a single determination, together with the means of the different genotypes, in calculating the theoretical frequency distributions. All assumptions necessary to the application of the formulas and procedures used in analyzing the data have been tested and proved correct. It is extremely important in the application of statistics to the analysis of biological data that the correctness of all assumptions necessary to the application of formulas be tested.

Effects and interactions of genes

From the genetic analysis made, it is clear that the theoretical means of the genotypes can be analyzed to determine the nature of the interactions of the genes with considerable confidence in the validity of conclusions drawn.

Some systematic method of presenting the main effects and determining the interactions and their nature is needed. POWERS (5) gave such a method for main effects that are regions of the chromosomes and generations. The method to be presented here for main effects that are pairs of genes is patterned after this method but differs somewhat because of the nature of the material being presented.

In table 11 the main effects are obtained by subtracting the mean of one genotype from that of another (table 9). For example, the main effect (0.054138) $Aa - AA$ under BB was obtained by subtracting the mean (0.598320) of the $AABB$ genotype from the mean (0.652458) of the $AaBB$ genotype. The other main effects were obtained in a similar manner.

The intra-allelic interactions under that heading in table 11 are differences between main effects. For example, the intra-allelic interaction $(aa - Aa) - (Aa - AA)$ under BB is the main effect $Aa - AA$ under BB subtracted from the main effect $aa - Aa$ under BB and is 0.299668 − 0.054138, or 0.245530, as

130568

listed in table 11. The other intra-allelic interactions were calculated in a similar manner.

Also, the interallelic interactions are differences between main effects. The interallelic interaction $Aa - AA$ under $Bb - BB$ is the main effect $Aa - AA$ under BB subtracted from the main effect $Aa - AA$ under Bb and is 0.118503 − 0.054138, or 0.064365. The same method was used in calculating all the interallelic interactions.

The intra-allelic–interallelic interactions are second- and third-order inter-

TABLE 11

MAIN EFFECTS AND INTERACTIONS OF GENES, TOGETHER WITH STANDARD ERRORS, FOR NUMBER OF LOCULES PER FRUIT (TRANSFORMED TO LOGARITHMS) FOR DANMARK × JOHANNISFEUER GROWN IN 1939

COMPARISON	DIFFERENCES		
	MAIN EFFECTS		
	BB	*Bb*	*bb*
Aa−*AA*	0.054138±0.021049	0.118503±0.011315	0.044977±0.011004
aa−*Aa*	0.299668±0.010701	0.220795±0.010097	0.203054±0.018074
	AA	*Aa*	*aa*
Bb−*BB*	0.059580±0.020552	0.123945±0.012194	0.045072±0.008233
bb−*Bb*	0.128540±0.010022	0.055014±0.012194	0.037873±0.016731
	INTERACTIONS Intra-allelic		
	BB	*Bb*	*bb*
(*aa*−*Aa*)−(*Aa*−*AA*)	0.245530±0.023613	0.102292±0.015165	0.158677±0.021160
	AA	*Aa*	*aa*
(*bb*−*Bb*)−(*Bb*−*BB*)	0.068960±0.022865	−0.068931±0.017245	−0.007199±0.018647
	Interallelic		
	Bb−*BB*	*bb*−*Bb*	
Aa−*AA*	0.064365±0.023897	−0.073526±0.015783	
aa−*Aa*	−0.078873±0.014713	−0.017141±0.020703	
	Intra-allelic–interallelic, second order		
	Bb−*BB*	*bb*−*Bb*	
(*aa*−*Aa*)−(*Aa*−*AA*)	−0.143238±0.028063	0.056385±0.026033	
	Aa−*AA*	*aa*−*Aa*	
(*bb*−*Bb*)−(*Bb*−*BB*)	−0.137891±0.028639	0.061732±0.025399	
	Intra-allelic–interallelic, third order		
	(*bb*−*Bb*)−(*Bb*−*BB*)		
(*aa*−*Aa*)−(*Aa*−*AA*)	0.199623±0.038279		

actions. The second-order interactions are differences between the first-order interaction differences. For example, the second-order interaction $(aa - Aa) - (Aa - AA)$ under $Bb - BB$ is the intra-allelic interaction $(aa - Aa) - (Aa - AA)$ under Bb minus the intra-allelic interaction $(aa - Aa) - (Aa - AA)$ under BB, or the interallelic interaction $aa - Aa$ under $Bb - BB$ minus the interallelic interaction $Aa - AA$ under $Bb - BB$. Either of these subtractions gives the intra-allelic–interallelic interaction value of −0.143238 listed in table 11.

The third-order interaction value is a difference between two second-order interactions. In table 11 it may be obtained by subtracting the second-order interaction −0.143238 from the second-order interaction 0.056385, or by subtracting the second-order interaction −0.137891 from the second-order interaction 0.061732.

The standard errors for the main effects and interactions listed in table 11 were calculated by application of the well-known formula for the standard error of difference.

The above discussion, together with the data presented in table 11, provides a model for deriving all the information pertaining to the main effects and interactions of two pairs of genes in such genetic studies. With the addition of each pair of genes, the derivation of all the information concerning the interactions rapidly becomes more complex. Usually the comparisons involving two pairs of genes at a time, regardless of the number of pairs of genes included in the study, will provide much of the pertinent genetic information.

An examination of the differences representing main effects in table 11 shows that in every case the means of the logarithms of number of locules per fruit for the Aa genotype exceed the means of the AA genotype, and the means of the aa genotype exceed the means of the Aa genotype. Also, in every case the means of the Bb genotype exceed the means of the BB genotype, and the means of the bb genotype exceed the means of the Bb genotype. For corresponding genotypes, the differences $Aa - AA$ for the three genotypes BB, Bb, and bb are not materially different from the differences $Bb - BB$ for the three genotypes AA, Aa, and aa. The differences $aa - Aa$ for the three genotypes BB, Bb, and bb are from two to five times the differences $bb - Bb$ for the three genotypes AA, Aa, and aa. Finally, as must follow from the results given immediately above, the differences $aa - AA$ for the genotypes BB, Bb, and bb are greater than the differences $bb - BB$ for the genotypes AA, Aa, and aa. The apparent similarity in effect of gene substitution in the comparisons $Aa - AA$ and $Bb - BB$ seems to be largely coincidental. Differences in dominance appear responsible for the results noted. As will be shown later, A exhibits a high degree of partial dominance over a in all genotypes, whereas the dominance relations between B and b are not consistent for the different genotypes. Clearly, substitution of a for A in all genotypes does not have the same effect upon number of locules per tomato fruit as does substitution of b for B in corresponding genotypes. It follows that these two pairs of genes are not duplicates.

The phenomenon of genic dominance involves an intra-allelic interaction of the genes, as has been pointed out previously by a number of workers (3, 5). If there is no dominance as regards the A and a genes, then $(aa - Aa) - (Aa - AA)$ should not be significantly different from

zero for any of the comparisons under intra-allelic interactions listed in table 11. All three of the differences are significantly different from zero, as in all cases they are more than six times their standard errors. *A* conditioning fewer locules per tomato fruit is at least partially dominant to *a* conditioning more locules per fruit. That *A* is not completely dominant to *a* is shown by the fact that the three differences for $Aa - AA$ given under main effects are significantly different from zero.

Similarly, if there is no genic dominance as regards the *B* and *b* genes, then the differences $(bb - Bb) - (Bb - BB)$ listed under intra-allelic interactions in table 11 should not be significantly different from zero. For AA and Aa the differences are statistically significant, as in each case they are at least three times the standard error. In the comparison for the *aa* genotype the difference is not significantly different from zero, as it is less than its standard error. Hence, as regards these data, *B* and *b* show no intra-allelic interaction for the *aa* genotype. For the AA genotype the difference $(bb - Bb) - (Bb - BB)$ is positive, showing that *B* is at least partially dominant to *b*. That dominance is only partial and not complete is shown by the fact that under main effects $Bb - BB$ for the AA genotype is 2.9 times its standard error. For the Aa genotype the comparison $(bb - Bb) - (Bb - BB)$ is negative, showing that in this genotype *b* is partially dominant to *B*. That it is only partially dominant is shown by the fact that the difference $bb - Bb$ under main effects for Aa is 4.5 times its standard error.

The data pertaining to the interallelic interactions are given in table 11. The first three values under interallelic interactions are at least 2.7 times their respective standard errors and therefore may be considered statistically significant. The fourth value, −0.017141, for $aa - Aa$ under $bb - Bb$ is not statistically significant, as it is smaller than its standard error. The first value is positive, and the remaining three are negative. It is apparent that there are interallelic interactions. The nature of these intra-allelic and interallelic interactions is of interest.

Both the difference $(aa - Aa) - (Aa - AA)$ and the difference $(bb - Bb) - (Bb - BB)$ are greater for the BB and AA genotypes, respectively, than for the Bb and Aa genotypes, and this difference is statistically significant in both cases. This shows that the degree of partial dominance of *A* over *a* is less in the Bb genotype than in the BB genotype. This tendency is carried to such an extent as regards the dominance relations between *B* and *b* that in the Aa genotype dominance is reversed, in that Bb more nearly approaches the behavior of bb than of BB in increasing number of locules per tomato fruit. As regards the bb and aa genotypes, the intra-allelic interactions of Aa and Bb are similar. The difference $(aa - Aa) - (Aa - AA)$ is greater in the bb genotype than in the Bb genotype but less than in the BB genotype. However, the difference noted for the bb and Bb genotypes is not statistically significant. Likewise, the difference $(bb - Bb) - (Bb - BB)$ is greater in the aa genotype than in the Aa genotype but less than in the AA genotype. These differences are statistically significant. The biological conclusion of genetic importance to be drawn from these data is that the degree of dominance as regards both *A* and *a* and *B* and *b* is dependent upon the genotype and that in case of *B* and *b* the phenomenon ranges from a high degree of partial dominance in association

with AA to no dominance in association with aa to dominance of b over B in association with Aa.

Also, the interallelic interactions are of interest. The difference $Aa - AA$ is greater in association with Bb than in association either with BB or with bb. Since the same interactions are involved, it follows that the difference $Bb - BB$ is greater in association with Aa than in association with either AA or aa. The behavior is not the same as regards the comparison $aa - Aa$ or $bb - Bb$. In this case the difference $aa - Aa$ is greater in association with BB than with Bb or with bb.

The intra-allelic–interallelic interactions of the genes are given by the second- and third-order interactions. From table 11 it can be seen that all the differences listed under second- and third-order interactions are statistically significant. The odds against the difference noted for the third-order interactions being due to chance are very great.

The intra-allelic interactions of the genes varied from a fairly high degree of partial dominance of one gene over its allele to partial dominance of the allele which in some genotypes had shown partial recessiveness. The interallelic interactions were such that the differences between contrasted pairs of genes were greater in some genotypes than in others. Finally, the data for the second- and third-order interactions show that the intra-allelic and interallelic interactions are dependent upon each other. The data are fairly conclusive in showing that the effect of gene substitution upon number of locules per tomato fruit varies according to the balance of the genotypic milieu.

It will be remembered that the theoretical means of the genotypes for 1940 were calculated from the theoretical means for 1939. The theoretical frequency distributions, calculated from these means, for the segregating populations gave a very good fit to the obtained frequency distributions for 1940. Hence the nature of the interactions of the genes for 1940 must be essentially the same as that for 1939. The 1940 data substantiate the conclusions drawn for the 1939 data concerning the nature of the interactions of the genes. In general, the conclusions drawn are supported by such a wealth of data as to leave little reason for questioning their validity.

Summary and conclusions

1. The interactions of the genes were such that no one scale of measurement could be found that was appropriate for describing the genetic variability.

2. The environmental variabilities were found to be following the logarithmic scale. This was true for all populations and genotypes. As shown previously (**6**), the environmental and genetic variabilities do not necessarily follow the same scale. Also, transformation of the individual plant data to logarithms did not equalize the environmental variances. For both years 1939 and 1940 the mean of Johannisfeuer was larger than either the mean of Danmark or the mean of the F_1, but the variance was smaller than the variance of either of these other two populations.

3. The interactions of the genes make it necessary to develop some method of estimating the theoretical means of the genotypes other than those given by POWERS *et al.* (**7, 8**).

4. The formulas developed for the purpose of estimating the theoretical means of the genotypes are $y = z - s_d x$ and $y = z + s_d x$, in which y is the estimated mean of the genotype under consideration, z is the upper limit of the class

from which the mean is being estimated, s_d is the standard error of a single determination for the genotype under consideration, and x is the x value of PEARSON's (4) table entitled "Area and Ordinate in Terms of Abscissa."

5. Whether end-classes or other classes of the frequency distribution are being used to estimate the means of the genotypes, usually the grouping should be such that at least ten plants fall in the class of the theoretical frequency distribution used for estimation.

6. In calculating the deductions to be made from the obtained frequency distributions to leave the percentage of plants of the genotype whose mean is being estimated, use is made of the theoretical rather than the obtained frequency distributions of the genotypes whose expected percentages are being deducted.

7. The following evidence establishes that Danmark and Johannisfeuer tomato varieties are differentiated by two pairs of genes. The differences between the obtained and the theoretical means of the three segregating populations are no greater than expected on the basis of random sampling. The fits between the obtained and theoretical frequency distributions are good, the theoretical frequency distributions having been calculated from the theoretical means of the genotypes based on the provisional genetic hypothesis and the environmental variances of these genotypes. Finally, the deviations between the obtained and theoretical genetic variances are no greater than expected by chance.

8. A model is given for deriving all the information pertaining to the main effects and interactions of two pairs of genes in such genetic studies. With each additional pair of genes the derivation of all the information concerning the interactions rapidly becomes more complex. Usually, the comparisons involving two pairs of genes at a time, regardless of the number of pairs of genes, will provide much of the pertinent genetic information.

9. The main effects of the two pairs of genes are not the same. The differences of $aa - AA$ are from two to three times the differences $bb - BB$.

10. There are intra-allelic, interallelic, and intra-allelic–interallelic interactions of the genes. The biological conclusion of genetic importance to be drawn from the intra-allelic interactions is that the degree of dominance, as regards both A and a and B and b, is dependent upon the balance of the genotype. In case of B and b the phenomenon ranges from a high degree of partial dominance in association with AA, to no dominance in association with aa, to dominance of b over B in association with Aa. Likewise, the interallelic interactions were such that the differences between contrasted pairs of genes were greater in some genotypes than in others. The second- and third-order interactions show that the intra-allelic and interallelic interactions are dependent upon each other. Finally, the data are conclusive in showing that the effect of gene substitution upon number of locules per tomato fruit in the Danmark × Johannisfeuer tomato hybrid varies according to the balance of the genotypic milieu.

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CYTOLOGY AND BREEDING BEHAVIOR OF TWIN PLANTS OF BROMUS INERMIS[1]

ETLAR L. NIELSEN[2]

Introduction

The study of twin plants of *Bromus inermis* Leyss. was undertaken to obtain more exact information regarding the reproductive processes in this species. It was hoped at the outset that twin sets of haploid-diploid chromosomal relations could be established, since plants characterized by the usually high polyploid numbers occurring in this species are investigated with considerable difficulty at best. Plants with low chromosome numbers might have greatly facilitated the study. The isolation of polyhaploids in mixed sets has not been realized.

The analysis of members of twin sets of plants is of both theoretical and practical interest. No information is available concerning the mode of origin of twin seedlings in smooth bromegrass. Twinning occurs approximately once in 550 seeds (unpublished data). However, plants producing as many as 8% of twin-embryoed seeds occur. If it is assumed that twin embryos arise from different embryo sacs, the plants investigated could as well have been siblings from a given plant. Conversely, if the embryos arose as a result of fertilization of two cells of a single female gametophyte, the two seedlings are of interest because the differences that occur between members of a given set of twins would be due to the genetic differences existing between the male gametes that effected fertilization. This would of necessity imply two pollen tubes reaching the female gametophyte to bring about fertilization. From the published reports covering macrosporogenesis and fertilization of other species, this would appear to be of rare occurrence. It has not been reported in any of the higher seed plants to the writer's knowledge. Likewise, it is not known whether other nuclei, such as the tube nucleus, can under certain extraordinary conditions effectively fertilize the polar fusion nu-

[1] Results of co-operative studies between the Division of Forage Crops and Diseases, U.S. Department of Agriculture, and the Department of Agronomy, Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

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cleus or another member of the developing female gametophyte. If this were possible and occurred, then only one pollen tube would need to reach the macrogametophyte. The resulting plants would be of similar genetic constitution. Several other modes of origin must be recognized that might be associated with twinning. These include: (*a*) one embryo may have arisen apomictically and the second by syngamy, with one of the resulting plants thus matroclinous in morphological and genetic characteristics, whereas the characteristics of the mate would be dependent upon the genetic constitution of the gametes involved, or (*b*) embryos may have arisen by zygotic cleavage. If they arose in the latter manner, the origin must be accompanied by other processes—for example, somatic segregation or mutation—since wholly similar sets of twins were not found.

During the progress of this study, and particularly as the data were being prepared for publication, it became evident that certain compiled observations should be considered even though they were not directly associated with the twin comparisons. To make them available to other workers in a separate publication would require repetition of much data included here. To avoid this, the data have been considered, first, as related to the analysis of the twin plants. Second, certain other general observations regarding the species conclude the presentation of the data. These observations are based upon the assumption that the plants studied constitute a random sample of smooth bromegrass as a whole.

There may be some objection to the above assumption because of the origin of these plants from multiple-embryoed seeds. These plants appear to be, however, sufficiently similar to the bulk of the population of *B. inermis* in morphological and breeding characteristics that representative conclusions are possible. Plants selected for detailed study represented the broad range of behaviors of the species in so far as was possible in the twin sets available.

This study does not purport to be a detailed cytogenetic analysis but rather was planned to gain insight into the general nature of cytological variability in the species. Such information was necessary to plan and execute detailed studies effectively. It is evident also that the behavior of this economically important species is such that it does not readily adapt itself to critical cytogenetic study.

Review of literature

Four different somatic chromosome numbers have been reported to occur in *B. inermis* from several sources. STÄHLIN (**24**) found 42 chromosomes in plants taken from near Obermenzing, Germany. More recently KNOBLOCH (**16**) examined twenty-seven plants of fifteen strains and selections of which five plants were reported to have 56, and twenty-two plants 42, chromosomes. The somatic count of 42 chromosomes has not been duplicated by other American workers.

Most somatic complements examined have been octoploid with 56 chromosomes (**2, 6, 9, 16, 19, 20**). One report by this writer (**19**) of 70 members in the somatic complement has been recorded, but, as HILL and MYERS (**13**) have indicated, plants of this constitution are no doubt rare in American material. A polyhaploid plant with 28 chromosomes was recently described by ELLIOTT and WILSIE (**10**).

KNOBLOCH (**16**) recorded that "late prophase chromosomes are all straight rods and during diakinesis they range in

length from 2.6 to 5.2 microns." Usually the metaphase plates contained only bivalents, but occasionally univalents occurred. Anaphase stages were irregular, but many of the tardy laggards reached the poles and were included in the telophase nuclei. Second-division figures were recorded to exhibit "regularity, but precocious as well as lagging anaphase separation may be observed."

More recent studies by ELLIOTT and LOVE (9) indicated that meiosis is quite irregular in the species. From 2 to 28 bivalents were observed as well as a relatively high frequency of multiple associations. There did not appear to be a close correlation of univalent and bivalent formation and multiple associations with normal quartets and pollen stainability. Subsequently, ELLIOTT (7) considered six species of the Bromopsis section of the genus. In *B. inermis* the correlation between stainable pollen and the percentage of normal pollen predicted from micronuclei analysis was −0.1. Meiosis in a polyhaploid plant of this species was relatively regular (10) in which averages of 1.1 quadrivalent and 0.09 hexavalent were found at diakinesis. Separation of these was almost normal as evidenced by rather low frequencies of multiple associations at metaphase I. Based upon the cytological behavior of this plant these workers suggested that there was a high degree of duplication at the octoploid level, that the high frequency of closed bivalents might be considered as an approach to autopolyploidy, and that segmental interchange had obscured to a large extent the original chromosomal condition. They recognized also that "forces other than homology might be responsible for the pairing complications."

Macrosporogenesis (20) has been recorded as following the "normal" pattern of development, with fertilization (18, 20) being effected 15–18 hours following anthesis. Post-fertilization development was studied by KNOBLOCH (17).

WALDRON (34) characterized the gross morphological features of some of the clones he had under test. He regarded the species as being highly cross-pollinated, a viewpoint opposed to that of KEZER (15). WALDRON's efforts to establish inbred lines were not successful (34). Several other workers reported findings (3, 4, 11, 14, 22, 31, 32) which are in agreement with those of WALDRON. SMITH (23) observed that, when reliable comparisons were possible, self- and cross-fertility appeared to be positively related. WALDRON (35) later gave a more detailed analysis of plants derived from two different sources. Correlation coefficients were determined for a number of relationships within and between populations.

ADAMS (1) found that seed-sets following controlled or open-pollination were not associated significantly with certain meiotic irregularities. It was concluded from an extensive series of diallelic crosses, self-pollinations, etc., that "the general level of fertility of each plant is influenced by additive gene action, whereas non-additive gene action appears to be causally related to specific cross-incompatibility effects . . . the type of gene action expected from the operation of oppositional factors."

ELLIOTT and LOVE (9), from their cytogenetic analysis of plants of this species, observed that "so much segmental interchange, presumably the result of natural selection, decreases the number of potential linkage groups and thus restricts recombination possibilities. . . . The meiotic irregularities present in some clones of smooth bromegrass introduce further limitations in obtain-

ing desirable gene combinations in inbred and cross-bred progenies."

ELLIOTT (8) has suggested that certain American forms of *B. inermis* with awned and pubescent lemmas were derived from the recombination of genes resulting from natural crossing with *B. pumpellianus* Scribn.

TABLE 1

SOURCES AND RELATIONS OF TWIN SETS OF B. INERMIS

Set	Source and history
5	Adventive colony in Bayfield County, Wisconsin
7	Synthetic strain of sister plants in I_2 derived from P.I. 101647 from Harbin, Manchuria
3	Adventive colony near Bayfield, Wisconsin
1, 2, 6, 27, 28	Adventive colony near Brodhead, Wisconsin
4, 25, 26	Adventive colony near Spooner, Wisconsin
9, 10, 16, 19, 22	I_1 plant[a] (from 195-5) from P.I. 101647 from Harbin, Manchuria
12, 14, 15, 17, 18, 20, 21, 23, 24	I_0 plant[b] (from 195-5) from P.I. 101647 from Harbin, Manchuria
8, 11	I_1 plant from P.I. 101647 from Harbin, Manchuria
13	I_0 plant from F.C. 22435 from Washington, Kansas

[a] Plant of first inbred generation.
[b] Plant derived following open-pollination.

Material and methods

Double-embryo seeds were observed in seed lots germinated in connection with other studies wherein the regular seed-laboratory procedures were employed. The twin-embryo seeds were removed from the germinating trays and placed in steam-sterilized soil in pots in a greenhouse where the seedlings were allowed to grow for approximately a week. They were then removed from the pots, and the roots washed free of soil. This delayed division of the seedlings was found most effective for successful isolation of the two without material damage to either. Earlier separations sometimes resulted in breakage and loss. The divided seedlings were then reset in individual pots and subsequently moved to the field. Twenty-eight sets of twins thus isolated were successfully grown to maturity. These form the basis of this paper, and their sources and pedigrees are included in table 1.

Characters whose evaluation is not suitable for exact measurement include vigor, color, coarseness or texture, disease reaction, etc. The data presented subsequently regarding these characters are based upon arbitrary classes established in connection with this and other investigations. Plant height was measured from ground level to the top of the panicles when the seed crop was ripe. Spread measurements are average diameters taken in two directions at right angles to each other through the middle of the colony. Seed weight was determined from duplicate lots of 100 seeds, where available, from each plant following open-pollination. Germination data are from duplicate lots germinated by use of standard seed-laboratory techniques. The number of spikelets per panicle was determined from actual counts of each of five typical panicles approximating those used for inclosure in selfing bags. The number of florets per spikelet was determined in ten spikelets chosen at random of each of the panicles selected for determination of spikelet number. Percentages of seed-set following open- and self-pollination were based upon the average florets per panicle. Five panicles were placed in each of three parchment selfing bags, and the number of seeds formed was determined following threshing. The percentage of florets setting seed following open-pollination was

based upon the average number of seeds set in the five panicles selected for spikelet determination.

The materials selected for cytological study were fixed in acetic-alcohol made of three parts of 95% alcohol and one part of acetic acid, or/and a modified Carnoy's fluid. All material examined in the meiotic studies was taken from field-grown plants. Approximately 24 hours after fixation the material was transferred to 70% alcohol and stored at 40° F. Acetocarmine smears were employed for the cytological studies reported. Some root tips were treated with a saturated solution of paradichlorobenzene prior to fixation in acetic-alcohol. Root tips were macerated by use of a 5% aqueous solution of ammonium oxalate at room temperature for approximately 2 hours before smearing.

The seedlings established for progeny studies were developed from seed following open- and self-pollination. Seed lots were germinated in individual small pans containing sterilized compost. The seedlings were reset into individual plant bands when about 2 inches tall and later transferred to the field. The usual cultivation and hoeing were employed to keep the field free from weeds and the soil in a good state of tilth.

Results

A. Morphological characteristics

Seedlings.—Differences were evident in certain characteristics of the seedlings, particularly such as vigor. This feature was perhaps directly related to position of the embryos with respect to the endosperm since original differences in vigor had nearly disappeared before the seedlings were set into the field. Other characters thought to be but slightly influenced by environmental conditions included texture or coarseness of the foliage and of the primary shoot, the presence and relative abundance of pubescence on the leaves and axis of the primary shoot, and the color of the latter near the soil. Leaves differed considerably in the manner in which they were carried upon the growing shoot: in some cases they were strict in aspect; in others they were lax and tended to droop perceptibly. Comparative observations are included in data given in table 2.

Twin seedlings were similar in general appearance but differed mostly in less obvious characters. For example, those of set 1 were alike in coarseness but differed in amount of pubescence, in color of bases of the primary axis, and in the aspects in which the leaves were carried. Seedlings of set 2 differed in all these characters—that designated as 2A was of coarse texture, moderately pubescent, and had purplish bases and lax growth in comparison with the medium texture, slight pubescence, green bases, and strict growth habit of 2B.

Coarseness was the principal difference evident between members of set 9—a feature that was strongly evident when the plants were mature. No discernible differences were evident between members of sets 16 and 19, and those of sets 13 and 18 were of small magnitude.

Adult plants.—Gross characteristics of adult plants studied are included in data summarized in table 3. Generally, there was considerable superficial similarity between members of most sets of twins. Although this relationship predominated, these data show that almost all plants differed in details.

Some sets of twins were closely similar, as illustrated in set 25 (fig. 1). The greatest variations occurred in reaction of these two plants to scald (*Rhyncosporium secalis* [Oud.] J. J. Davis), in pubescence

upon lemmas, and in germinability of seeds produced. They differed somewhat in number of spikelets per panicle, in florets per spikelet, and in seed weight (table 5). Essentially this same relationship was apparent also in sets 7, 8, 13, 14, 17, and 23. The major variations between the members of set 7 were in number of spikelets per panicle and in seed weight. The lemmas of 8A were pubescent, whereas those of 8B were smooth or glabrous. Plant 8B had more spikelets per panicle and heavier seed. Evidence indicated that 13B was more susceptible to brown spot (*Pyrenophora bromi* Died.) than 13A. It also carried more spikelets per panicle, more florets per spikelet, and produced somewhat heavier seed than 13A. Inflorescences of 14A possessed fewer spikelets per panicle, but more florets per spikelet than those of its mate, 14B. Seed weight and the percentage of germination (table 5) were essentially similar, however. There was a difference in weight of 140 mg. per 100 seeds between those produced by the members of set 17. These observations indicate that even though some twin plants appear superficially similar, they are often quite different in certain characters.

Some of the remaining twin sets dif-

TABLE 2

SOME CHARACTERISTICS OF TWIN SEEDLINGS OF B. INERMIS

Set	Texture[a]		Pubescence[b]		Base[c]		Other[d]	
	A	B	A	B	A	B	A	B
1	Md	Md	Sl	M	G	P	E	L
2	C	Md	Md	Sl	P	G	L	E
3	MF	F	Sl	O	P	G	L	E
4	F	MC	Md	O	P	P+	.....	.....
5	Md	F	Sl	O	P	G	.....	.....
6	C	Md	M	Sl	LP	P	.....	.....
7	Md	F	M	Sl	G−	P	.....	.....
8	Md	VF	Sl	Sl	LP	P	.....	.....
9	F	Md	O	O	P	P	W	S
10	Md	C	O	O	G	LP	.....	.....
11	C	C	M	M	LP	G	.....	.....
12	Md	F	M	Sl	LP	VLP	.....	.....
13	Md	M	Sl	VSl	LP	LP	L	E
14	VF	MC	O	O	P	G	W	S
15	Md	F	Sl	M	LP	P	.....	.....
16	Md	Md	O	O	P	P	.....	.....
17	F	Md+	O	O	LP	DP	.....	.....
18	Md	Md+	M	M	LP	P	.....	.....
19	Md	Md+	O	O	P	P	.....	.....
20	Md	F	M	M	LP	P	.....	.....
21	MF	Md	Sl	M	DP	G	.....	.....
22	F	Md	O	O	P	P	.....	.....
23	F	Md	O	O	DP	MdP	.....	.....
24	C	Md	O	O	G	R	.....	.....
25	C	Md	O	O	G	G	.....	.....
26	F	Md	O	O	P	SlR	.....	.....
27	Md	F	O	O	P	DP	S	VW
28	Md	Md	Sl	M	GR	P	.....	.....

[a] Texture: C = coarse; Md = medium; F = fine; VF = very fine.

[b] Pubescence: O = none; VS = very slight; Sl = slight; Md = medium; M = dense.

[c] Seedling base (color): G = green; P = pink; LP = light pink; DP = dark pink; R = red; GR = green red.

[d] Other: E = erect; L = lax; W = weak; S = strong; VW = very weak.

fered considerably both in general appearance and in their less obvious characteristics. Members of set 9 were similar with reference to plant height, spread, vigor, and leafiness. Their appearance was strikingly different (fig. 2) owing primarily to the wide, dark bluish-green leaves of 9A as compared with the medium-wide leaves of the more intermediate and usual green color of 9B. The latter also possessed more spikelets per panicle, but these were made up of fewer florets than were those of 9A. The weights of 100 seeds of this twin set were 320 and 210 mg. for 9A and 9B, respectively. The heavier seeds were somewhat higher in germinability.

Certain features of set 11 suggested close relationship in morphological characters. For example, the plants were essentially similar in height, spread, flowering date, panicle habit, number of florets

TABLE 3

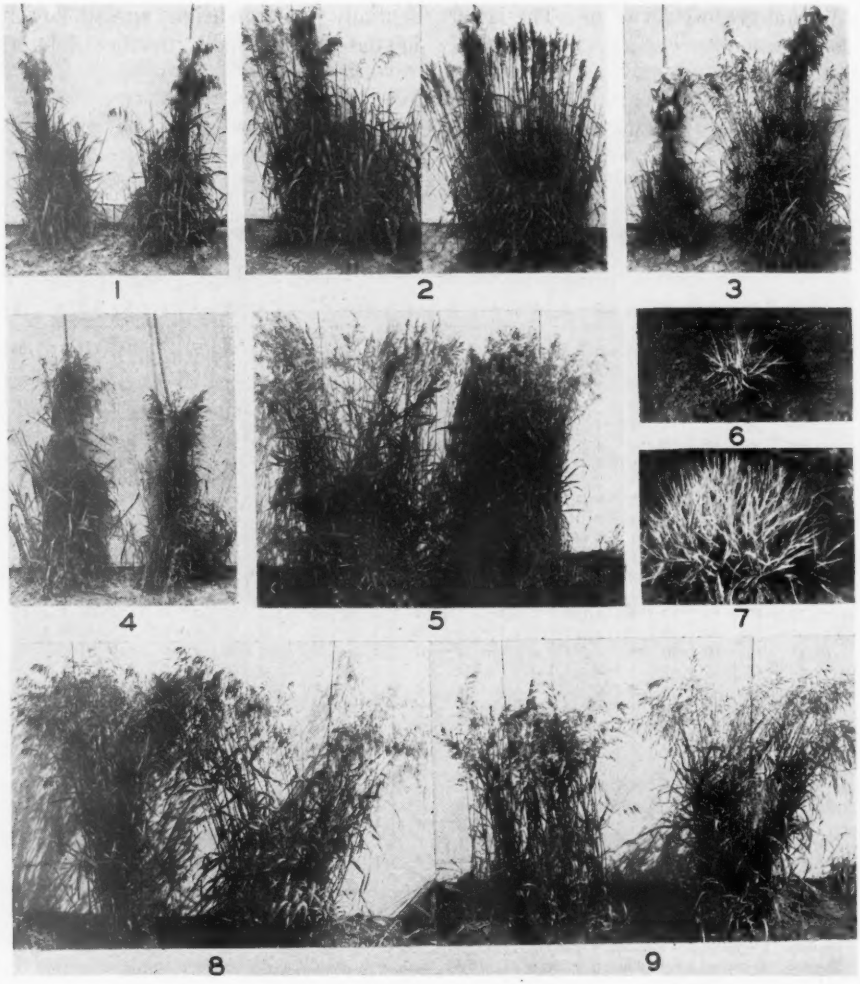
SUMMARY OF MORPHOLOGICAL CHARACTERISTICS OF ADULT PLANTS OF TWIN SETS OF B. INERMIS

Set	Height[a]		Spread[a]		Vigor[b]		Leafiness[c]		Texture[d]		Panicle habit[e]		Difference in flower[f]	Awns[g]		Pubescence[h]		Spikelets/panicle		Florets/spikelet	
	A	B	A	B	A	B	A	B	A	B	A	B		A	B	A	B	A	B	A	B
1....	32	34	16	18	3	3	2	1	2	2+	1	1	2	1	3	0	2	41	77	4.7	5.0
2....	39	36	24	22	2	2	2	3	2	2	1	2	2	1	1	2	0	67	55	5.1	3.7
3....	45	39	17	15	2	3	1	2	2	2	1	2	0	1	1	1	0	57	53	7.0	6.3
4....	37	38	24	18	2	3	2	1	2	2	2	2	2	1	1	0	0	76	52	4.5	6.9
5....	40	39	25	18	2	4	3	3	1	1−	2	2	3	4	0	0	0	51	53	4.8	4.9
6....	44	39	25	18	2	4	2	2	2−	1	1	2	0	3	1	0	0	65	49	5.2	4.9
7....	39	40	16	22	2	2	2	2	1−	1−	2	2	0	0	0	0	0	75	56	6.3	6.0
8....	39	38	21	15	2	2	1	1	2	2+	1	1	0	1	1	3	0	49	58	5.2	5.2
9....	42	39	15	18	2	2	3	3	1	2	1	2	1	3	4	0	0	71	96	6.6	5.2
10....	39	38	17	26	2	2	2	1	2+	1−	2	2	2	1	4	3	0	56	50	4.6	5.5
11....	39	42	31	20	1	2	1	2	2	1−	2	2	0	3	3	0	1	66	54	5.5	5.2
12....	42	37	21	23	2	3	2	2	2+	3+	2	1	3	0	0	3	2	57	79	4.6	5.0
13....	37	39	16	20	3	3	3	2	2	2	1	1	1	0	0	0	0	55	69	5.0	6.1
14....	46	51	26	19	1	1	1	2	2	1	2	1	0	0	0	0	0	43	54	8.2	6.0
15....	37	40	21	20	2	2	2	3	1−	1+	1	2	0	2	0	0	0	42	68	7.9	6.8
16....	40	31	26	21	2	4	2	1	1+	3	2	1	5	0	3	0	0	46	48	6.3	5.7
17....	41	43	16	19	2	1	1	2	1−	1−	1	1	0	3	3	2	0	72	77	5.5	6.0
18....	42	35	25	17	1	2	1	2	2	1	1	2	0	0	4	3	0	74	53	5.6	6.0
19....	30	24	15	12	4	4	1	1	3	3	2	2	0	1	1	0	0	52	63	4.5	5.4
20....	41	39	18	19	3	2	2	1	1	2	2	1	0	0	0	0	3	80	68	6.0	5.1
21....	48	46	17	24	2	2	3	2	1	1	2	1	2	0	1	0	0	52	80	5.0	5.8
22....	37	38	17	19	3	3	1	2	2	1	1	1	0	1	3	0	0	67	95	5.8	5.0
23....	30	28	16	12	4	4	1	1	3	3	1+	1+	0	3	1	2	0	38	32	5.0	4.0
24....	49	46	15	12	2	2	3	3	2+	1	1	1	3	3	0	0	0	58	41	5.4	6.0
25....	35	36	12	11	4	4	2	2	2	2	1	1	0	1	1	0	3	45	38	6.8	7.2
26....	37	28	18	8	2	4	1	1	2	3−	1	1	2	1	0	3	3	47	68	5.3	4.5
27....	36	40	15	11	3	3	3	3	2+	1	1	1	0	3	3	3	3	70	74	4.3	4.5
28....	30	41	9	12	4	3	2	3	2−	1−	1	1	1	1	3	3	3	61	54	4.7	5.1

[a] Height and spread in inches.
[b] Vigor: 1 = very good; 2 = good; 3 = fair; 4 = poor.
[c] Leafiness: 1 = leafy; 2 = medium; 3 = stemmy.
[d] Texture: 1 = coarse; 2 = medium; 3 = fine.
[e] Panicle habit: 1 = lax; 2 = strict.
[f] Days difference in flowering between plants A and B.
[g] Awns: 0 = none; 1 = long; 2 = medium; 3 = short; 4 = bristle.
[h] Pubescence on lemma: 0 = none; 1 = on nerves; 2 = slight between nerves; 3 = much between nerves.

per spikelet, and the short-awned lemmas. Characters contrasting the two plants included leafiness and texture, which caused them to appear quite different. Plant A of this combination was considerably more susceptible to *Pyrenophora*, carried more spikelets per panicle, and produced somewhat lighter seeds that were markedly inferior to those of plant B in percentage of germination.

Large differences occurred between most characteristics of the members of set 16, plant A being vigorous, moderately leafy, very coarse, and of lax panicles. Plant B was 5 days later in coming into flower and was more sus-



FIGS. 1–9.—Twin sets and albinotic plants of *B. inermis*. The A member of twin sets is on right in figs. 1, 3, and 4, on the left in figs. 2, 5, 8, and 9. Fig. 1, set 25; fig. 2, set 9; fig. 3, set 26; fig. 4, set 28; fig. 5, set 24; figs. 6 and 7, albinotic plants in I_1 progeny of plant 26A; fig. 8, set 2; and fig. 9, set 4.

ceptible to brown spot. Some difference occurred also in the number of spikelets per panicle and of florets per spikelet. Plant A was highly sterile, whereas 16B produced rather light seed of inferior germinability.

The most diverse twins successfully separated and grown to maturity were those of sets 26 (fig. 3) and 28 (fig. 4). A difference of approximately 10 inches occurred both in plant height and in spread between the two members of set 26. The two plants were essentially similar in leafiness, panicle habit, and pubescence on lemmas. Material variations occurred in all other characters studied. Members of set 28 were of essentially the same pattern, although they were not so extreme in morphological characteristics as were those of set 26. However, plant 28A was sterile to self-pollination, while 28B was somewhat fertile.

The examples cited are illustrative of characteristics of individual twin sets. More complete comparisons can be made by inspection of data in table 3, which indicate the nature of variation found to occur between members of twin sets. Adult plants, as well as seedlings, were not detectably different from plants originating from single-embryo, or typical, seeds. The variations, major as well as minor, occurring in these plants are representative of those generally found in any population of the species derived from open-pollinated seed.

B. Reproductive characteristics

Data in this section relate to reproductive processes and include observations regarding pollen diameter and abortion, seed-set following self- and open-pollination, seed weight and germinability, and chromosome number and meiotic behavior.

Pollen

Pollen diameter.—Average diameters were determined from IKI-stained pollen. Twenty-five grains were measured for each plant. With one exception, only grains showing a typical reaction to iodine were selected for measurement; otherwise, diameters were determined from pollen grains selected at random. The fifty-six plants fell into three broad classes with respect to pollen size (table 4). One class, characterized by diameters averaging 84.6–88.0 μ, contained only sets 2 and 4. One set of twins (set 24) had small pollen that averaged 49.2 μ in diameter. The measurements for this set are not entirely comparable with those for other sets, since no fertile pollen was found in approximately forty-five anthers from fifteen florets from different panicles. Necrosis was evident in pollen of these plants shortly after the exine was formed. Hence, it was decided that, since the pollen measurements would not be strictly comparable otherwise, those measured should be of equivalent age, that is, taken approximately at the time of anthesis of the plants with which they were compared. The empty shells, or exines, thus constitute the sample studied. It appeared likewise that empty grains of other plants exhibited approximately the same average range and size as did the stainable pollen. The third class of plants with pollen diameters of intermediate size (61–78 μ) included members of twenty-five of the twenty-eight sets of twins examined. Generally, there was some variation in pollen diameters of a given plant, a characteristic doubtless related to the preceding irregularity during the meiotic divisions.

Pollen abortion.—The percentage of aborted pollen in a given plant was determined from forty random fields of the anther contents stained with IKI. The

TABLE 4

PERCENTAGES OF ABORTED POLLEN AND χ^2 FOR DIFFERENCE BETWEEN PLANTS IN POLLEN STERILITY; AND POLLEN DIAMETER AND F VALUES FOR BETWEEN-MEMBER-OF-SET VARIATION OF TWINS OF B. INERMIS

Plant	Aborted		Diameter		
	Percentage	Chi square[a]	Range	Average	F[a]
1A	2.9		68– 80	73.4	
1B	12.6	92.3**	60– 76	69.2	11.73**
2A	6.8		80– 96	88.0	
2B	3.1	38.3**	68– 96	85.0	2.29
3A	100.0		60– 68[b]	63.9[b]	
3B	22.6	**	60– 84	68.8	9.77**
4A	4.4		76– 96	85.0	
4B	4.8	0.3	64–100	84.6	0.02
5A	99.3		60– 72[c]	66.0	
5B	96.5	21.2**	64– 80	72.2	18.82**
6A	10.9		60– 76	69.2	
6B	2.8	91.9**	64– 72	68.0	1.07
7A	5.8		56– 68	62.8	
7B	8.5	6.1*	56– 68	62.8	0.00
8A	10.9		64– 72(92)[d]	69.8	
8B	1.3	121.6**	68– 84	74.0	5.60*
9A	84.1		72– 80	76.2	
9B	94.1	51.1**	64– 76	70.2	18.61**
10A	21.0		64– 84	72.2	
10B	1.8	336.9**	60– 76	69.4	5.54*
11A	13.4		60– 76	68.0	
11B	11.5	2.8	60– 76	67.4	0.19
12A	11.1		60– 72	66.0	
12B	3.9	51.3**	60– 72	66.4	0.12
13A	17.0		60– 80	69.0	
13B	50.7	438.8**	60– 80	68.6	0.05
14A	11.9		60– 76	68.6	
14B	6.2	31.6**	60– 68	64.6	10.71**
15A	19.9		64– 92	77.8	
15B	39.8	141.3**	68– 84	76.8	0.31
16A	76.6		60– 72	65.8	
16B	99.9	376.6**	64– 80	73.3[e]	25.63**
17A	16.1		60– 72	65.8	
17B	19.9	9.9*	64– 80	70.6	10.51**
18A	12.0		64– 72	68.6	
18B	9.6	5.5*	60– 76	68.2	0.13
19A	24.1		68– 88	77.6	
19B	12.3	100.2**	68– 84	77.0	0.13
20A	5.3		64– 76	70.2	
20B	36.0	609.5**	60– 80	73.0	3.45
21A	13.5		60– 72	62.6	
21B	6.6	37.0**	56– 72	61.0	1.68
22A	5.8		64– 76	69.2	
22B	35.5	518.2**	60– 68	63.6	15.56**
23A	0.6		60– 72	67.6	
23B	1.4	40.7**	68– 80	74.6	20.42**
24A	100.0		44– 52	49.2[f]	
24B	100.0	0.0	44– 52	49.2[f]	0.00
25A	4.6		64– 86	76.6	
25B	10.7	35.7**	64– 76	69.6	14.52**
26A	9.4		64– 80	67.8	
26B	9.1	0.1	60– 72	67.4	0.10
27A	10.7		68– 80	74.8	
27B	11.3	0.3	72– 84	76.2	2.11
28A	3.5		60– 68	64.0	
28B	2.0	5.9**	72– 80	76.0	32.26**

[a] * = Significant at the 5% level; ** = significant at the 1% level.

[b] Based upon exines of empty grains.

[c] Based mostly upon exines of empty grains.

[d] One large grain, 92 μ in diameter.

[e] Based upon six fertile grains found.

[f] Approximately fifteen slides were made from as many florets of different spikelets. No fertile pollen was found on any slide. The number of grains was not counted.

number of grains examined for a given plant varied from 848 to 3146, with from 1200 to 2000 grains observed for most plants (table 4). Any pollen grain exhibiting a definite reaction to iodine was classified as functional. Empty and typically brownish grains were classed as nonfunctional. Size was not considered in relation to pollen abortion, though many of the larger or smaller grains were perhaps nonfunctional.

The lowest percentage of pollen abortion found to characterize a given plant was 0.56% (plant 23A), whereas several plants were regarded as completely sterile (3A, 16B, 24A, 24B). Generally, there was some diversity with respect to pollen fertility between members of a given twin set. Members of sets 4 and 26 were closely similar, differing from each other by less than 0.5% in frequencies of aborted pollen. The greatest difference occurring within sets, 77.4%, was in set 3, one member of which was totally sterile. The data were analyzed statistically, and the chi-square and F values for differences in pollen abortion and in pollen size are included in table 4. The percentages of aborted pollen were not significantly different between twins in five sets and were significantly different in three sets, whereas differences between members of each of the remaining twenty sets were all highly significant.

Seed-set and quality

Cross-fertility.—Cross-fertility is used here to denote seed set following open-pollination. Percentages of florets setting seed following open-pollination were relatively low in most plants studied (table 5). Over 50% of the florets of members of twin sets 1, 2, 24, 25, and 27 formed seed. Members of set 1 were the most cross-fertile, with 95 and 72% of the florets of plants A and B, respectively, being fertile. Respective percentages for plants A and B of sets 2 and 25 were essentially similar, that is, 64 and 91% for the former as compared with 66 and 91% for the latter. The members of twin combinations 24 and 27 were somewhat lower, with percentages of 63 and 61, and 63 and 52, for the A and B plants, respectively. There were five other sets including 6, 10, 21, 26, and 28 wherein the percentage of cross-fertility was at least 50% for one member, whereas that of the mate was somewhat lower. The seed-set of three additional sets, 12, 22, and 23, approached 50%.

Low cross-fertility was apparent in the members of set 3 (16.0 and 5.6%), set 7 (3.6 and 9.8%), and set 16 (5.2 and 12.9%). The most cross-sterile plants considered were 5B and 15B, wherein only 0.2 and 0.3% of the florets subsequently produced seeds.

A consideration of these data for seed-set following open-pollination reveals also that both members of one set fell into each of the following fertility classes: 0.0–9.9% (set 7); 20–29% (set 9), and 60–69% (set 24). Two sets fell into each of the 30–39% (sets 4 and 20) and the 40–49% classes (sets 12 and 22). Members of the remaining twenty-one sets differed in their percentages of cross-fertility. Differences separating the two plants were relatively small in some instances (sets 3 and 5), while larger differences occurred between others (sets 2, 10, 14, 15, 26, and 28). For example, 3.3% of the florets of plant 28A set seed compared with 86.6% of those of its mate. Likewise, 15A was 41.1% cross-fertile compared with 0.3% in 15B. These examples indicate extremes evident in behavior of this characteristic and illustrate similarities and dissimilarities found in members of twin sets in this species.

SELF-FERTILITY.—An analogous examination of the data concerning self-fertility indicated that members of thirteen twin sets fell into similar fertility classes. These include set 7 (0.01–0.04% class); sets 3 and 5 (0.05–0.09% class); sets 8, 10, 13, 17, 21, 22, and 23 (0.1–0.99% class); and sets 1, 6, and 27 (1.0–9.9% class). In self-fertility, as in cross-fertility, differences occurring between members of some sets were relatively small (sets 9, 16, 19, and 20) and between others, quite large (sets 2, 25, 26, and 28).

SEED QUALITY.—The weights of 100 seeds were essentially the same in members of some sets, as in 12, 13, 14, 25, and 27 (table 5). The greatest difference found between members of a single set (18) was 200 mg.—a difference approximating the average weight of the lightest of the two seed lots compared. Other sets showing large differences in this character were 9, 10, 17, 22, and 24.

As shown, seed weights of the members of set 18 were quite different. In this instance the lighter seed was of inferior germinability—73% as compared with 91% for the mate. Variations in germinability were also apparent in the seed of sets 20, 21, 24, 25, and others. Generally,

TABLE 5

PERCENTAGES OF FLORETS SETTING SEED FOLLOWING SELF- AND OPEN-POLLINATION, AVERAGE WEIGHT OF SEED (MG/100 SEEDS), AND PERCENTAGES OF GERMINATION OF OPEN-POLLINATED SEED OF B. INERMIS

PLANT	PERCENTAGE SEED-SET FOLLOWING		WEIGHT (MG.)	GERMINATION (%)	PLANT	PERCENTAGE SEED-SET FOLLOWING		WEIGHT (MG.)	GERMINATION (%)
	Open-Poll.	Self-Poll.				Open-Poll.	Self-Poll.		
1A	95.4	1.18	365	96	15A	41.1	0.11	420	94
1B	71.5	2.14	405	97	15B	0.3	0.03	.....	.....
2A	63.7	0.44	425	97	16A	5.2	0.03	.....	.....
2B	90.6	8.94	405	95	16B	12.9	0.00	260	73[b]
3A	16.0	0.05	440	96	17A	17.6	0.29	275	92
3B	5.6	0.05	360[a]	92[b]	17B	21.9	0.46	415	96
4A	30.4	0.08	370	94	18A	21.2	0.09	410	91
4B	37.9	3.95	315	95	18B	8.9	0.04	210	73
5A	14.4	0.08	305	97	19A	19.6	0.00	310	96
5B	0.2	0.08	.....	.....	19B	22.3	0.04	230	85
6A	61.9	1.94	450	98	20A	34.6	0.10	285	91
6B	47.3	1.72	405	94	20B	31.2	0.09	330	84
7A	3.6	0.04	260[a]	.....	21A	61.5	0.12	250	81
7B	9.8	0.04	320[a]	78[a]	21B	48.6	0.13	330	91
8A	25.3	0.18	310	95	22A	49.4	0.20	305	86
8B	45.7	0.27	345	95	22B	41.1	0.85	420	92
9A	26.5	0.01	320	93	23A	45.9	0.92	345	91
9B	20.8	0.00	210	84	23B	37.4	0.47	370	73[b]
10A	50.4	0.90	380	96	24A	63.2	0.28	325	84
10B	10.3	0.32	280	92	24B	61.0	0.00	470	95
11A	11.3	0.06	310	79[b]	25A	66.3	18.10	380	93
11B	32.9	0.19	340	93	25B	90.9	1.36	365	84
12A	44.1	0.00	265	86	26A	75.0	1.41	380	93
12B	47.1	0.07	250	88	26B	33.2	0.07	285	86
13A	32.9	0.24	385	92	27A	63.1	3.59	350	93
13B	28.9	0.11	405	95	27B	52.2	9.77	350	98
14A	11.6	0.00	330	86	28A	3.3	0.05	.....	.....
14B	42.5	0.10	345	87	28B	86.6	0.60	265	97

[a] Weights based upon duplicate samples of twenty-five seeds.

[b] Based upon a single sample of twenty-five seeds.

however, the seed was of high germinability, and the differences of small magnitude.

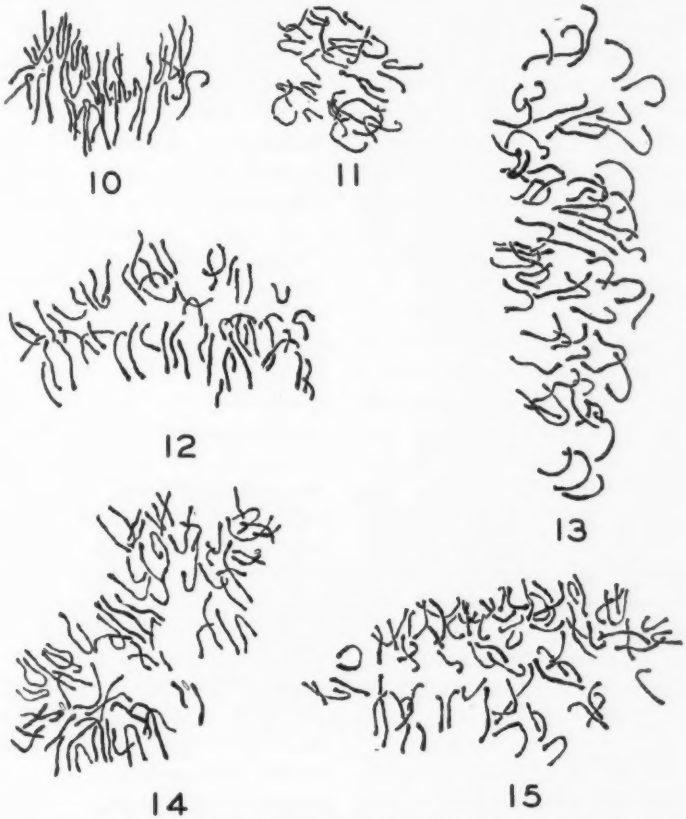
Cytology

Chromosome number.—Three different chromosome numbers occurred in the material examined, these being 28, 56, and 70 in somatic complements. Not all combinations of twins were examined, however, since pollen diameters of the remaining twin sets fell into the size range characterized by the 56-chromosome races examined.

Twenty-eight chromosomes were present in root-tip cells of members of set 24 (figs. 10, 11). Except for number of members in the complement, no other characteristics defined these plants as being markedly different in their chromosomal features from other plants of this species having higher chromosome counts. The chromosomes were mostly long and rod-, U-, or V-shaped.

Fifty-six chromosomes characterized sixteen twin sets, of which only those of set 1 are shown (figs. 12, 13). Definable differences did not appear between members of the sets with respect to chromosome morphology.

The somatic count of 70 chromosomes



Figs. 10–15.—Camera lucida drawings of somatic complements in plants of *B. inermis*. Fig. 10, plant 24A; fig. 11, plant 24B; fig. 12, plant 1A; fig. 13, plant 1B; fig. 14, plant 2A; fig. 15, plant 2B.

had been in doubt until two sets of twins (2 and 4) characterized by this number were isolated (figs. 14, 15). The chromosomes of these complements were essentially similar in their morphological characteristics to those of plants with lower numbers. Those of plant 2B appeared to be somewhat shorter than those of its mate, but the difference is of doubtful significance. Three small fragments occurred in the somatic complements of plant 2A (fig. 14), as well as in several others of the twins studied.

General morphological features of these adult plants with 28 and 70 somatic chromosomes are shown in figures 5, 8, and 9. These can be compared with figures 1–4 of plants possessing 56 chromosomes in their somatic complements.

MEIOTIC BEHAVIOR.—The number of observations regarding meiotic behavior were limited somewhat in the interest of examining a reasonably adequate number of plants. Accordingly, the data compiled relate to the frequency of laggards during successive stages of development. Data are summarized in table 6. For convenience they are arranged in the following manner: two twin sets (sets 5 and 9) from which no inbred progenies were derived (except a single plant from 5A) are considered first. This is followed by data from five sets of twins from which inbred progenies were developed from one plant, whereas none was obtained from the mate of the set, owing either to complete self-sterility or to a few weak plants that did not survive. The remaining ten sets of twins constitute those wherein both members were moderately self-fertile, several to many seeds resulting from self-pollination.

Plants of twin set 5 differed to a certain extent in the nature of their meiotic divisions. Average frequency of laggards was somewhat greater in plant A than in its mate. Laggards were in evidence in well over one-half of the sporocytes at metaphase I. Twenty per cent and 40% of the sporocytes of plants A and B were free of such bodies, respectively. There is a characteristic rise in frequency of discernible laggards at anaphase I, a feature no doubt related to the easier interpretation of figures at this stage. An inversion bridge with an accompanying acentric fragment was occasionally observed in sporocytes of 5B, whereas a bridge without a fragment occurred in 0.04% of the cells of plant A. There is a regular decline in number of laggards per sporocyte from this stage through the quartet stage. Two bridges, one with an acentric fragment, were observed in telophase I cells of plant 5A. Frequency of micronuclei at the quartet stage in plant A was roughly twice as great as that observed for plant B. Both members of the set were highly pollen sterile, 99.3 and 96.5% for plants A and B, respectively. Members of this set were quite infertile following both self- and open-pollination.

The pattern of the divisions of set 9 essentially paralleled that of plant 5A. Most sporocytes of these plants were characterized by laggards at all stages of development from metaphase I through the quartet. Throughout the divisions 9B was somewhat more regular than its mate, but a higher percentage of its pollen aborted. Percentages of fertile florets following open-pollination were 26.5 and 20.8, respectively, for plants A and B. Both plants were essentially self-sterile.

The second group of five sets of twins examined included those where one was self-sterile, as determined by self-pollination, and the mate somewhat self-sterile. It constituted a rather heterogeneous group with respect to the meiotic divisions of its members. The earlier phases of the divisions, including meta-

phase I and telophase I, were essentially similar in sets 14, 18, and 24 and were only somewhat less irregular than those of sets 5 and 9 considered above. Members of sets 16 and 19 were somewhat different in the behavior of the individual plants. Plant 16A was characterized by being quite irregular at anaphase I and had a greatly decreased number of laggards at the subsequent stages. Seven micronuclei were present per one hundred cells at the quartet stage, but the

TABLE 6

AVERAGE FREQUENCY OF LAGGARDS AT METAPHASE I, ANAPHASE I, AND TELOPHASE I, AND OF NUMBER OF MICRONUCLEI AT DYAD AND QUARTET STAGES, AND PERCENTAGE OF NONSTAINABLE POLLEN IN TWIN PLANTS OF B. INERMIS

Plant	Metaphase I		Anaphase I				Telophase I				Dyad		Quartet		Pollen
	Av. lag.	% free	Av. lag.	% free	b[a]+f[a]	b[a]	Av. lag.	% free	b[a]+f[a]	b[a]	Av. micro.	% free	Av. micro.	% free	% non-stainable
5A	3.23	20	4.24	12	.....	0.04	2.13	4	0.04	0.04	0.96	0	0.94	0	99.3
5B	1.20	40	3.48	8	.04	.....	1.28	24	.....	.....	.66	20	.52	12	96.5
9A	3.92	8	5.36	4	.28	.....	3.80	16	.....	.....	.98	28	1.53	0	84.1
9B	1.44	40	3.56	16	.40	.....	1.88	20	.....	.....	.80	28	.89	20	94.1
14A	1.48	24	2.56	12	.16	.....	2.00	16	.....	.04	.86	8	.66	12	11.9
14B	1.08	32	1.24	32	.12	.08	.80	44	.....	.04	.42	18	.15	68	6.2
16A	.84	40	2.20	8	.....	.....	.84	48	.04	.....	.42	40	.07	84	76.6
16B	2.24	24	.56	0	.....	.....	2.88	20	.24	.....	.54	28	.44	16	99.9
18A	1.12	48	1.84	28	.....	.....	1.80	12	.....	.....	.70	32	.72	8	12.0
18B	1.32	20	2.40	16	.....	.....	2.04	20	.16	.04	.82	20	.46	24	9.6
19A	.56	64	1.44	36	.28	.08	1.16	28	.04	.....	1.00	42	.28	36	24.1
19B	1.40	48	1.24	40	.12	.04	1.20	36	.04	.....	.32	56	.18	72	12.3
24A	1.48	32	2.92	24	.12	.04	1.52	24	.04	.....	.56	20	.38	28	100.0
24B	1.76	20	2.80	4	.44	.....	1.88	16	.....	.....	.58	24	.77	12	100.0
4A	1.64	24	1.92	36	.04	.16	.76	60	.....	.....	.20	76	.14	72	4.4
4B	.44	68	1.48	24	.08	.04	.88	36	.....	.....	.32	48	.14	56	4.8
17A	1.60	20	2.48	8	.04	.....	2.20	16	.08	.....	.72[c]	28	.45	24	16.1
17B	1.84	16	1.88	12	.20[b]	.....	.88	36	.08	.....	.54	44	.26	64	19.9
20A	1.44	28	2.00	40	.04	.....	1.52	12	.....	.....	1.16	6	.39	24	5.3
20B	1.76	32	4.20	12	.....	.....	2.40	0	.....	.....	.92	20	.93	8	36.0
26A	.76	56	1.40	40	.08	.....	.64	52	.04	.....	.26	68	.03	88	9.4
26B	.56	64	1.36	44	.12	.....	.76	52	.....	.....	.20	64	.13	72	9.1
1A	3.48	24	1.28	28	.40	.20	.48	36	.20	.24	.42	40	.33	32	2.9
1B	.92	56	1.56	24	.16	.20	.44	60	.....	.....	.14	72	.11	56	12.6
2A	1.32	40	1.00	44	.20	.....	.92	28	.02	.....	.84	36	.84	56	6.8
2B	1.00	52	2.80	20	.16	.....	1.20	28	.02	.....	.80	52	1.80	24	3.1
7A	1.68	20	2.96	8	.12	.....	2.16	16	.....	.....	.34	52	.56	28	5.8
7B	1.00	28	3.20	0	.....	.....	1.96	8	.40	.04	.52	44	.38	28	8.5
11A	.60	68	1.04	52	.16	.04	.44	72	.....	.....	.20	72	.05	84	13.4
11B	.80	64	2.00	36	.....	.12	.40	80	0.04	.04	.26	68	.09	72	11.5
23A	.28	76	1.00	64	.....	.20	1.56	28	.....	.....	.32	60	.17	60	.6
23B	2.44	12	2.48	24	.....	.04	1.48	20	.....	.....	.60	28	.15	60	1.4
25A	.60	60	1.72	28	.20	.08	.68	56	.....	.....	.34	52	.13	60	4.6
25B	.88	44	1.24	48	0.04	0.12	.92	48	.....	.....	0.40	40	0.32	36	10.7

[a] b = bridge; f = fragment.

[b] Excluding two cells with numerous bridges, not countable.

[c] Excluding one bridge between cells.

pollen was highly abortive, with approximately 77% being nonstainable. Seed-sets following open- and self-pollination were 5.2 and 0.03%, respectively. An average of almost three laggards occurred in telophase I division figures of 16B. Irregularity was somewhat less at subsequent stages, however. Sixteen per cent of the members of the quartets were free of micronuclei. Almost all the pollen was nonfunctional, and only 13% of the florets were fertile following open-pollination and none following self-pollination. Essentially this same pattern of meiotic divisions occurred also in members of set 19. A larger percentage of pollen of plants of this set was iodine-positive, with 24.1 and 12.3% of the grains being abortive in plants A and B, respectively. Plant A was completely sterile following self-pollination compared with 0.04% fertility in plant B.

Sufficient seed was set following self-pollination in the remaining ten sets of twins to permit establishment of inbred progenies of varying sizes from all plants. Some progenies were composed of relatively few plants, however. Several features were apparent in data compiled from members of this group that were not evident in those previously considered. These include somewhat less irregularity throughout successive stages of the meiotic divisions, greater percentages of stainable pollen, and an increased seed-set following both open- and self-pollination. Members of sets 17 and 20 were the least regular of the plants included in this group. Although there was a definite decline in number of laggards evident during successive stages of meiosis, a moderately high frequency of micronuclei remained at the quartet stage, the frequency of these varying from 0.26 to 0.93 per cell. These were generally distributed throughout most of the quartet cells, since only 8% of the cells of quartets of plant 20B, and 24% of those of plants 17A and 20A, were free of such bodies. Percentages of nonstainable pollen varied from 5.3 to 36. Less than 35% of the florets of these plants set seed under open-pollination.

Members of sets 11 and 26 were the most regular meiotically of the paired plants examined. Excepting at anaphase I, all were characterized by having an average of less than one laggard at each stage. Thirteen per cent and 11% of the pollen of the A and B members of set 11 failed to give a positive iodine reaction compared with approximately 10% in members of set 26. However, only one of these plants was markedly fertile under open-pollination. Seventy-five per cent of the florets in plant 26A were fertile compared with approximately one-third of the florets of plants 26B and 11B, and 11% of 11A. Plant 26A was the most fertile of these four plants following self-pollination, with 1.4% of the florets developing seeds compared with 0.07% in its mate. Corresponding percentages for plants A and B of set 11 were 0.06 and 0.19, respectively.

Members of sets 1 and 2 were the most fertile of the plants considered under conditions of open-pollination. Members of both sets were characterized by moderate frequencies of inversion configurations at anaphase I and telophase I. Laggards were somewhat more common in 2B than in other members of these twin combinations. At the quartet stage 0.33 and 0.11 micronuclei were in evidence in plants A and B of set 1, respectively. They were somewhat higher in set 2, where the frequencies were 0.84 and 1.8. Fertility was mostly satisfactory following both open- and self-pollination.

Further observations can be made regarding the heterogeneity that occurs

within each group considered with respect to meiotic behavior. The greatest variation between plants at metaphase I occurred in set 1, wherein there was a difference of 2.56 laggards between the members of the set. The sporocytes of plant 23B possessed 2.16 more laggards at this stage than did its mate. The smallest difference at this stage was in sets 11 and 26, wherein a difference of 0.2 laggard characterized the two sets of twins while the members of set 25 differed by only 0.3. Although plants considered in the lower fertility group were somewhat more irregular than those of other groups, smaller differences usually prevailed between members of a given set at metaphase I.

At anaphase I plant 20A had 2.00 laggards compared with 4.20 in the mate. Members of set 26 were essentially similar in frequency of laggards, being separated by 0.04, a difference of doubtful significance. Sets 19 and 24 were also closely similar: the former set differed by 0.2 and the latter by 0.12 laggards.

The largest difference separating members of a set at telophase I was 2.04 laggards in set 16, of which one plant was sterile and the mate somewhat fertile following self-pollination. This is compared with 3.80 in plant 9A and 1.88 in 9B. Several sets of twins were quite similar in behavior at this stage. These included sets 1, 11, and 19, wherein there was a difference of 0.04. Members of set 23 were separated by 0.08 laggard compared with 0.12 between members of sets 4 and 26.

Smaller differences occurred in the frequency of micronuclei at the dyad stage than characterized the earlier stages in their frequencies of laggards. The largest variation observed occurred in the members of set 19, which were separated by 0.68 micronucleus. The twins of set 5 differed by 0.30 micronucleus, sets 1 and 23 by 0.28, set 14 by 0.44, and set 20 by 0.24. Several other sets were characterized by small differences. The smallest of these was 0.02 micronucleus in the members of set 24, followed by 0.04 in set 2 and 0.06 in sets 11, 25, and 26.

The largest difference occurring in the frequency of micronuclei at the quartet stage was in members of set 2. Plant A had 0.84 micronucleus per quartet cell compared with 1.80 in plant B. Members of set 4 did not differ in frequency of micronuclei, and small and perhaps insignificant differences of 0.02 occurred in set 23, 0.04 in set 11, and 0.10 in set 19. There appeared to be a definite trend at the quartet stage, and to a somewhat lesser extent at the dyad stage, for differences in magnitude to be inversely related to the extent of self-fertility. This trend was also evident in pollen fertility and in seed-set following open-pollination.

Percentages of aborted pollen were most variable in the class of twins wherein one member was self-sterile and the mate somewhat fertile following bagging. A difference of 23% separated the members of set 16. Small and insignificant differences occurred in the frequency of aborted pollen in members of sets 4, 23, and 26, all of which are included in the higher fertility class. The greatest difference found between members of twin sets was 31%, in set 20, which was generally characterized by moderate self-fertility.

Further comparisons are possible between combinations represented by difference chromosome numbers. Set 24 is included in the class of which one member was somewhat fertile following self-pollination and the mate sterile. The class as a whole is highly variable in its meiotic behavior, with the tetraploid

plants of set 24 tending to be somewhat more irregular than the octoploids.

Both sets of decaploid twins (2 and 4) fell into the higher self-fertility class. These were more regular in their divisions than members of other combinations during the earlier division stages. Pollen abortion averaged about 6% less than in other plants of the class; this suggests that the somewhat higher mean frequencies of micronuclei at the dyad and quartet stages may have been due to sampling error. They were also more fertile than the remainder of the class, which were either octoploid or presumed to be octoploid because of their pollen diameters, following both open- and self-pollination.

C. Progeny behavior

Heading during year of establishment.—The tendency for certain plants to develop fruiting culms during the year of establishment appears to be an inherited characteristic when observed in general grass-breeding nurseries. This tendency was relatively weak in progenies of some twin plants—for example, nine of the thirty-seven plants in the progeny following open-pollination of plant 1A developed fruiting culms during their first season under field conditions. The twelve plants included in the I_1 generation of this plant failed to head during the year of establishment. Similarly nine of the thirty-three plants in the I_0 progeny of 27B developed fruiting culms compared with one of thirty-three plants derived following self-pollination. However, highly significant differences occurred in the frequency of this characteristic in the I_0 generation of sets 4, 7, and 22, and significant differences between the members of sets 21 and 27. Differences approaching significance occurred between members of sets 9 and 28.

In the I_1 generation the only significant differences between progenies of twins with respect to first-year heading occurred in those derived from sets 11 and 23. The differences between inbred progenies grown from sets 4, 10, and 13 approached significance. Thus set 4 was the only one wherein this tendency was expressed in the progenies derived following either bagging or open-pollination.

Effects of inbreeding.—Effects of inbreeding in the I_1 generation were determined in two ways. Clones from parent plants were established with progenies developed from seed produced either following open- or self-pollination. These plants, with their progenies, were classified according to arbitrary vigor classes with "1" designating exceptionally vigorous plants and "6" the very weak members of the progeny. Plants of intermediate vigor were classified in the intervening classes. These observations were made after plants had reached their maximum development following anthesis, and their seeds were approaching ripening. Plant height was also used as an index of vigor. Measurements were made of maximum plant height when seed ripened. The two methods of determination were in close agreement. Since plant height readily adapts itself to comparative discussion, the data so derived will be considered.

Inbred progenies were not obtained from nine of the plants considered. Twenty-one members of twin sets yielded from one to five adult plants, seven produced from six to ten, nine from eleven to twenty, and ten from thirty-one to forty. There were no progenies in the twenty-one to thirty-plant class. The average plant heights for the paired progenies derived from seed resulting from open- (Op) and self-pollination (S) are used in the following discussion as an expression

of the relation of the inbred- to the Op-progeny, here referred to as the S/Op percentage.

The data in table 7 show that wide differences may occur between members of twin sets as expressed by reduction in vigor as a result of one generation of inbreeding. Since this is amply and more reliably demonstrated by the relative behaviors of the larger I_1 progenies, these will be considered most fully. The average plant heights of the I_1 progenies of the members of twin set 6 were 97.2 and 98.0%, respectively, of their I_0 counterparts (figs. 16, 17). Similarly, the I_1 generation of sets 1, 27, and 28 were approximately 90% as vigorous as the corresponding I_0 progenies. A reduction in vigor of about 14 and 18% occurred in the I_1 generation from the two members of set 17; the actual difference between the two was 3.6%.

Members of set 25 were essentially similar in vigor and morphological characteristics. Average height of plants of the I_1 generation from 25B was 95.6% of that of its paired Op-progeny. This contrasts sharply with the 71.9% for plant A of the set; the difference in this case was 23.7% (figs. 18, 19). Even larger differences of 40.3 and 31.9% occurred between plants of the I_1 generations from members of sets 15 and 20, respectively. The latter two illustrations are, however, based upon very small I_1 progenies wherein the occurrence of an exceptionally vigorous or a relative weak plant had a disproportionate effect upon the S/Op percentage.

D. General considerations

It has been possible to make certain general observations regarding the plants studied which are considered representative of smooth bromegrass—irrespective of their origin as twins.

Adult plants.—Differences in intensity and nature of foliage color have been referred to in twin comparisons. Color differences were apparent also at the nodes. Most were of several different shades of brown. Green-noded plants were somewhat less common, while plants with reddish nodes were quite rare.

Large variations occurred in the amount, position, and nature of the pubescence at the culm nodes exceeding the flag leaf. In some instances the indumentum was completely absent, whereas in others it occurred both above and below the nodes. When present, it was usually more abundant below than above the nodes. Occasionally it was evident as a fine puberulence which resulted in a downy appearance of the structure. Generally, the pubescence is fine and acutely retrorse in aspect. In one twin plant (26B) it tended to assume a pilose character.

Some variation was apparent also in the panicles. The number of spikelets per panicle in the plants examined ranged from thirty-two to ninety-five, while the number of florets per individual spikelet fluctuated between four and eight.

The lemmas were significantly different in their amount of pubescence as well as in width. Some lemmas were devoid of pubescence, on some it was found only upon the nerves, and in others it was present in different amounts on the faces of the lemmas between nerves. Occasionally a trace was present, while at other times the indumentum was copious, particularly near the callus. Generally, the rachilla possessed some pubescence, although glabrous individuals were relatively frequent.

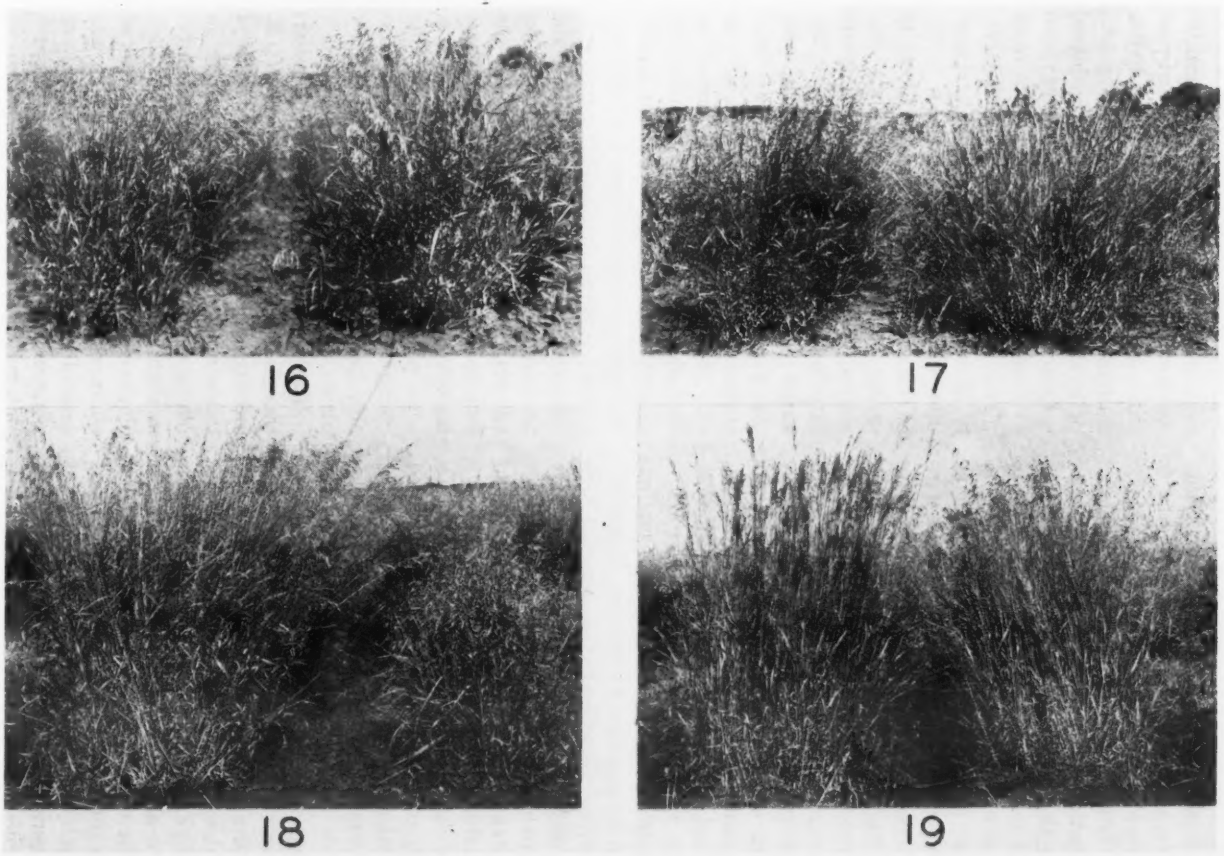
Awns as long as 2.5 mm. were relatively common. This structure, when present, varied greatly. It was often ab-

TABLE 7

NUMBER OF PLANTS IN PROGENIES OF B. INERMIS DERIVED FROM SELF- (S) AND OPEN-POLLINATION (OP), THEIR MEAN HEIGHTS IN INCHES, AND RATIO OF I_1 TO I_0 PROGENY EXPRESSED IN PERCENTAGE OF THE OP

Plant	N	Average height	S/Op	Plant	N	Average height	S/Op
1A S.	12	38.2		15A S.	2	52.5	
Op.	37	41.8	91.4	Op.	39	48.6	108.0
1B S.	35	42.8		15B S.	2	32.5	
Op.	34	46.0	93.0	Op.	6	48.0	67.7
2A S.	8	40.3		16A S.	1	28.0	
Op.	36	47.4	97.7	Op.	30	42.8	65.4
2B S.	40	41.0		16B S.	0		
Op.	35	45.5	90.1	Op.	35	44.6	
3A S.	2	49.5		17A S.	12	36.2	
Op.	37	43.3	114.3	Op.	38	44.0	82.3
3B S.	3	41.8		17B S.	19	36.7	
Op.	33	41.3	101.2	Op.	37	42.7	85.9
4A S.	2	37.5		18A S.	4	44.3	
Op.	35	44.5	84.3	Op.	39	43.5	101.8
4B S.	37	43.2		18B S.	0		
Op.	39	45.4	95.2	Op.	29	46.7	
5A S.	1	45.0		19A S.	0		
Op.	37	45.4	99.1	Op.	37	39.8	
5B S.	0			19B S.	5	22.2	
Op.	2	51.0		Op.	31	32.1	69.2
6A S.	39	47.8		20A S.	2	50.5	
Op.	39	49.2	97.2	Op.	38	46.3	109.1
6B S.	31	49.1		20B S.	4	36.3	
Op.	38	50.1	98.0	Op.	32	47.0	77.2
7A S.	3	51.0		21A S.	1	54.0	
Op.	36	47.2	108.1	Op.	39	46.8	115.4
7B S.	1	45.0		21B S.	6	48.8	
Op.	36	48.8	92.2	Op.	39	49.0	99.6
8A S.	1	45.0		22A S.	8	37.4	
Op.	35	46.9	95.9	Op.	37	42.3	88.4
8B S.	6[a]	41.2		22B S.	33	38.8	
Op.	39	47.5	86.7	Op.	37	40.6	95.6
9A S.	0			23A S.	18	43.3	
Op.	38	45.6		Op.	38	42.8	101.2
9B S.	0			23B S.	7	35.4	
Op.	37	43.7		Op.	39	41.4	85.5
10A S.	18	39.7		24A S.	10	45.8	
Op.	39	46.2	85.9	Op.	38	48.1	95.2
10B S.	6	41.8		24B S.	0		
Op.	32	45.1	92.7	Op.	38[a]	50.4	
11A S.	2	48.0		25A S.	32	30.9	
Op.	39	43.7	109.8	Op.	36[b]	43.0	71.9
11B S.	3	45.7		25B S.	35	45.2	
Op.	38	46.0	99.3	Op.	37	47.3	95.6
12A S.	0			26A S.	20[c]	42.1	
Op.	37	43.5		Op.	37	48.5	86.8
12B S.	4	43.8		26B S.	17	42.1	
Op.	37	46.2	94.8	Op.	37	43.9	95.9
13A S.	3[a]	40.3		27A S.	31	45.1	
Op.	38	43.8	92.0	Op.	36	48.8	92.4
13B S.	7	43.5		27B S.	33	40.9	
Op.	37	43.8	99.3	Op.	33	45.1	90.7
14A S.	0			28A S.	2	35.5	
Op.	40	46.8		Op.	9	38.9	91.3
14B S.	3	56.0		28B S.	18	40.5	
Op.	39	53.1	105.5	Op.	29	45.1	89.8

[a] Excluding one not heading.
[b] Excluding three not heading.
[c] Excluding four not heading.



FIGS. 16–19.—Inbred (I_1 *right*) and open-pollinated (I_0 *left*) progenies from twin plants of *B. inermis*. Fig. 16, from 6A; fig. 17, from 6B; fig. 18, from 25A; fig. 19, from 25B.

sent. Upon some lemmas it occurred as a bristle-like elongation of the midrib, whereas in others it attained lengths as great as those indicated above.

Data in table 4 indicate the large variations among plants in percentages of functional pollen as ascertained by the IKI-reaction. The range was from approximately complete fertility to complete pollen abortion. No doubt some smaller and larger pollen grains are nonfunctional even though iodine-positive in reaction.

Percentages of florets setting seed following open- and self-pollination varied greatly also. Although a considerable range of variation occurred in the plants studied, the average fertility approximated 37% following open-pollination. This apparently low average fertility is not unexpected, since critical examination of panicles at random in general breeding nurseries has shown that high frequencies of empty florets are commonly encountered. On the contrary, these percentages of seed-set may be considered relatively satisfactory, since it is shown elsewhere that complex cytological irregularities are commonly encountered in microsporocytes.

SEED-SET.—Several plants were relatively self-fertile as compared with the usual seed-set following self-pollination. It can be accepted that these plants are naturally highly self-fertile and that the seed-set obtained was not due to faulty technique. An effort was made to eliminate all foreseeable factors that might cause error in the results. This included the use of new parchment selfing bags which were placed before the onset of flowering and checked for damage each forenoon so that damaged individuals, if any, could be replaced before the afternoon pollen shed. Selfing bags and their contents were harvested and placed in separate kraft bags which remained intact until threshing, when the seed-set was determined. Thus, every effort possible was taken to eliminate possible sources of error. Seed-set following self-pollination as a whole was considered satisfactory for the establishment and maintenance of inbred lines from most plants. Loss of lines can be kept effectively at a minimum by an adequate sampling of the population.

Data for cross- and self-fertility showed some inconsistency when considered in relation to the percentages of functional pollen. There was, for example, rather close agreement between the percentages of cross-fertility and stainable pollen. Certain plants, such as 5A, 5B, and 16B, were characterized by low percentages of stainable pollen. In these the percentage of seed-set following open-pollination was low also but somewhat better than might be expected. This was probably closely related to the favored physiological status of the developing female gametophyte and to the availability of functional pollen. There occurred, however, wide discrepancies in some plants between seed-set following open-pollination and percentage of stainable pollen. Illustrative of these are plants 3B, 4A, and 8B.

The data are somewhat similar, though less obvious, with respect to self-fertility. Relatively satisfactory seed-set following self-pollination occurred in plants characterized by high and intermediate frequencies of stainable pollen. The seed-set following self-pollination of plants characterized by poor pollen was generally quite low, although there were some exceptions to this generalization.

SEED QUALITY.—Marked differences occurred also in the seed weights resulting from open-pollination. Average weights of 100 seeds varied from 210

to 470 mg. Generally, however, germination was quite satisfactory, though some exceptions were found. Percentage of germination was closely correlated ($P < 0.01$) with the seed weight of plants investigated.

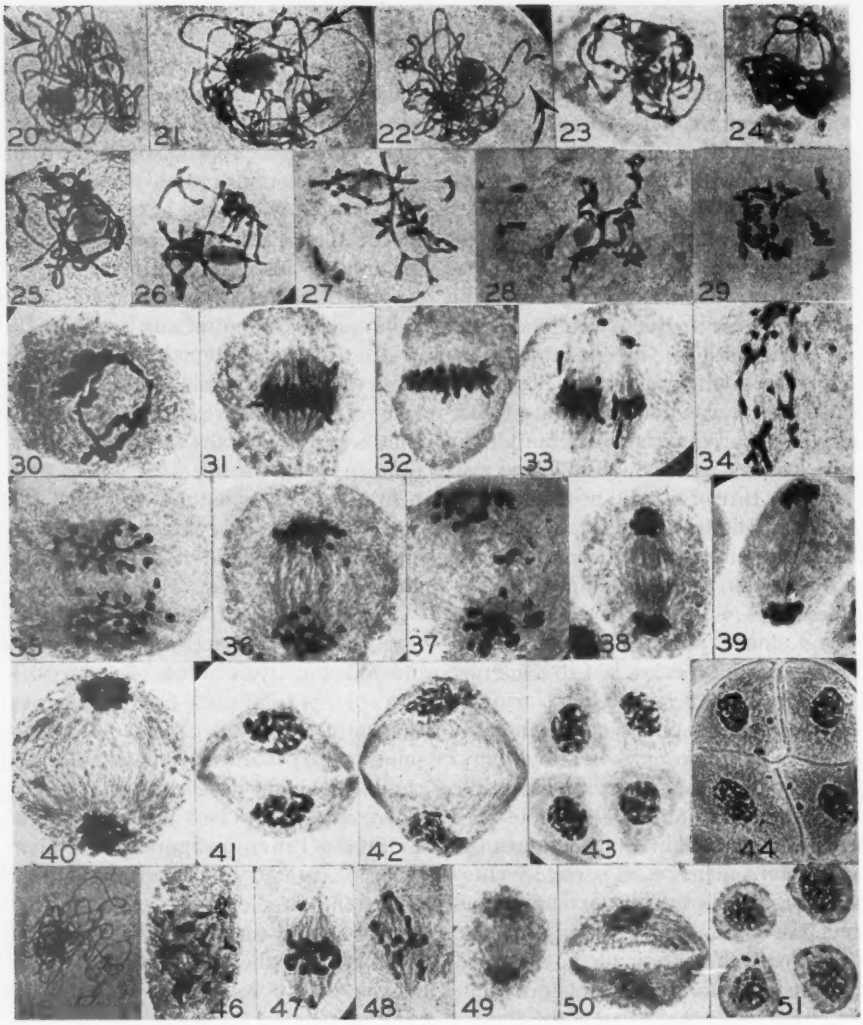
MEIOTIC BEHAVIOR.—A striking feature of the prophase stages of meiotic divisions in smooth bromegrass is the relatively high frequency of translocation configurations. Obviously, with the large number of chromosomes present in the species it was not possible to follow individual chromatids throughout their entire lengths; invariably they were soon lost in the thread mass. The usual translocation configurations were in evidence near the outer fringes of the figures at the pachytene and diplotene stages of development (figs. 24, 26). True translocations are no doubt present in considerable numbers in the species, but it also must be recognized that a goodly portion might be due to the duplication of whole or portions of chromosomes in a high polyploid species such as *B. inermis*. Multiple associations of 4, 6, and 8 chromosomes were observed, and in some instances the entire complements were joined by chromatic or sticky threads extending between the chromosomes (figs. 25, 27–30).

The loop characterizing an inverted segment in a chromosome was occasionally observed; a majority of these were no doubt overlooked in the thread mass. In chromosomes of some plants there was evidence of poor homology as indicated by poorly paired threads. Achromatic segments were observed in several plants (fig. 22). In some instances the segments were quite short, whereas others were of considerable length, as is apparent in the figures referred to. It was not possible to locate the affected chromosomes in root tips treated with paradichlorobenzene. Deletions were rather commonly observed as indicated by rather short unpaired "loops" along the chromatids in otherwise paired relationship (figs. 20, 23). Some of the sporocytes of one plant wherein deletions were evident were characterized also by having a single-strand thread associated with a deletion. In another instance a rather long, unpaired thread was commonly observed in prophase configurations (fig. 21).

Multiple associations were commonly observed at diakinesis, these being the rule rather than the exception. Generally they were so complex that analysis was difficult. The members of entire complements were sometimes involved (fig. 30). In some sporocytes apparent associations were considered to be due to stickiness rather than to catenations.

The metaphase I stages observed were usually very compact, a condition that precluded analysis in most instances. It was evident from these figures, however, that some included chains of chromosomes (fig. 31) and that both open and closed bivalents were present (fig. 32). There appeared to be a tendency for the metaphase I division figures of one plant to be orientated in two groups on the plate, the smaller one being approximately a third the size of the larger (fig. 33). Likewise, there was a tendency evident in plant 19B for several of the chromosomes to form a second plate at metaphase I. Usually the secondary plate was made up of 3–5 chromosomes.

Anaphase I and telophase I figures were usually complex also. However, some anaphase I figures were not so badly bunched as were the configurations of some of the other stages (figs. 36–40). An interesting feature observed occasionally throughout the plants studied, and rather commonly in a few plants, was the presence of "chains or multiple associations" apparently passing to the



FIGS. 20–51.—Photomicrographs of meiotic divisions and their products in *B. inermis*. Fig. 20, prophase with deletions, plant 20A; fig. 21, prophase with unpaired chromatid, plant 20A; fig. 22, prophase with achromatic segment, plant 20A; fig. 23, diplotene with deletions, plant 26A; fig. 24, diplotene with translocation configuration; fig. 25, diplotene with stickiness, plant 2A; fig. 26, late diplotene with translocation configuration, plant 1B; fig. 27, early diakinesis with multiple associations and stickiness; fig. 28, diakinesis, plant 1B; fig. 29, diakinesis, plant unknown; fig. 30, diakinesis in ring configuration; fig. 31, metaphase I with members of multiple associations off plate, plant 26B; fig. 32, metaphase I with open and closed bivalents, plant 26A; fig. 33, metaphase I with divided plate, plant 9A; fig. 34, anaphase I with stickiness, plant 9A; fig. 35, anaphase I with stickiness, plant 23A; fig. 36, anaphase I, plant 1A; fig. 37, anaphase I with dividing lagging univalents; fig. 38, telophase I with chromosome bridge without acentric fragment, plant 1A; fig. 39, telophase I with chromosome bridge and the acentric fragment adjacent to (out of focus) lower short arm; fig. 40, telophase I, plant 23A; fig. 41, dyad with chromosome bridge, plant 26A; fig. 42, dyad, plant 24B; fig. 43, quartet, plant 2A; fig. 44, quartet with micronuclei, plant 26B; fig. 45, prophase, plant 24A; fig. 46, diakinesis, plant 24A; fig. 47, metaphase I, plant 24B; fig. 48, anaphase I, plant 24B; fig. 49, late anaphase I, plant 24A; fig. 50, dyad, plant 24B; fig. 51, quartet, plant 24A.

poles as a unit. These are apparent in several of the photographs (figs. 34, 35). Chromatin fragments were frequently observed, particularly at telophase I and subsequent stages (fig. 44).

Some quartets gave an indication of poor reorganization of the chromatic material. A poorly defined membrane was apparent when member cells of the quartets were in an early stage of collapse. Likewise, this and the dyad stages were sometimes characterized by the presence of fragments or small micronuclei. In other cases the excluded bodies were relatively large, thus resulting in considerable variation in sizes of those bodies (figs. 40–44).

Among other peculiarities observed was the occurrence of microsporocytes of two distinct sizes in the member plants of twin set 24. Comparable stages of development of sporocytes are shown in figures 45–51. It had been observed repeatedly in sporogenous tissue from single anthers of these plants that large sporocytes, essentially similar in size to those found in octoploid plants, were interspersed with their smaller counterparts. Aside from noting the occurrence of this peculiarity, the problem of their origin has not as yet been given serious attention.

Certain generalizations are possible from the data compiled and summarized in table 6. For example, the four plants making up the first group considered, that is, the self-sterile plants, were characterized by a high frequency of laggards throughout their meiotic divisions. The average frequency of laggards at the successive stages were: metaphase I, 2.4; anaphase I, 4.2; telophase I, 2.3; dyad, 0.9; and quartet, 1.0. The pollen was generally of low viability, since only 6.7% of it gave a positive iodine reaction. The average seed-set of the four plants was 15.5% and 0.04% following open- and self-pollination, respectively.

The second group of plants listed in table 6 is made up of twin sets of which one member was fertile and the other sterile following self-pollination. Regarding these plants as a unit, the average frequency of laggards and micronuclei fell into an intermediate position. Self-sterile plants of this class were, as expected, more closely similar in their regularity of divisions, their percentages of nonstainable pollen, and their seed-set to the sterile plants of the first class considered, whereas the self-fertile plants were more similar in these regards to the most self-fertile group.

Plants of the third class (i.e., those from which moderate-sized inbred progenies were derived) had a lower average frequency of laggards than those of the classes previously referred to, excepting at anaphase I. At this stage the average frequency of laggards was essentially the same in the two more self-fertile classes. The mean frequency of laggards in the self-fertile class was: at metaphase I, 1.25; at anaphase I, 1.95; and at telophase I, 1.13. Dyads had 0.5 micronucleus per member cell, whereas in the quartets there was an average frequency of 0.37 micronucleus per cell. Approximately 9.4% of the pollen was nonfunctional compared with 93.5% in the self-sterile class. Comparable mean percentages of seed-set were 45.1 and 2.02 in self-fertile plants as contrasted with 15.5 and 0.04 in plants of the self-sterile class.

These data concerning meiotic behavior, pollen fertility, and seed-set were further classified upon an individual plant basis rather than as combinations of plants. The data were summarized in five classes according to the percentage of fertility following self-pollination (table 8).

The eleven plants included in the 0.0–0.04% fertility class have the highest average frequency of laggards and micronuclei per sporocyte of the several classes. Likewise, they are characterized by the lowest average frequency of functional pollen.

The average frequency of laggards throughout the successive stages was inversely, and that of laggard-free sporocytes positively, correlated with the reduced seed-set following open-pollination of this plant may perhaps be explained by sampling error, since only one plant is included in the class.

PROGENY BEHAVIOR.—The data regarding progeny behavior (table 7) were also considered upon an individual plant basis. Nine of the fifty-six plants studied failed to set seed following self-pollination. Twenty-one plants yielded first-generation inbred progenies of from one to five plants. Nine of these I_1 progenies were at least as vigorous as the paired I_0 progenies which resulted from open-pollinated seed. The I_1 generation from five plants was characterized by a severe to moderate inbreeding depression; these had S/Op percentages of 65.4, 67.7, 69.2, 77.2, and 84.3. The vigor of the progenies of the seven remaining plants was from 91.3 to 99.9% of that of their mates. It would appear from these illustrations that low self-fertility of a plant is not necessarily associated with exceptional vigor of the plants derived therefrom, since in this case the two classes appear to be about equally divided.

From six to ten plants were included

TABLE 8

AVERAGE MEIOTIC BEHAVIOR AT SUCCESSIVE STAGES OF DEVELOPMENT, AND PERCENTAGES OF POLLEN ABORTION AND SEED-SET IN B. INERMIS ACCORDING TO FERTILITY CLASSES

No. plants	Self-fertility class (percentage)	Metaphase I		Anaphase I		Telophase I		Dyad		Quartet		Pollen abortion (%)	Seed-set	
		No. laggards	% free	No. laggards	% free	No. laggards	% free	No. micronuclei	% free	No. micronuclei	% free		Op	Self
11	0.0 – 0.04	1.60	30.5	2.57	13.0	1.98	22.2	0.65	33.6	0.57	30.2	47.9	18.4	0.02
7	0.05– 0.09	1.49	41.1	2.59	26.9	1.35	32.0	.56	41.1	.48	38.9	38.4	20.3	.08
9	0.1 – 0.99	1.36	35.6	1.89	31.6	1.25	32.0	.60	34.2	.32	50.7	18.6	40.1	.36
6	1.0 – 9.99	1.21	51.3	1.62	31.0	.78	43.3	.38	51.8	.47	46.0	7.2	76.9	3.17
5[a]	1.0 – 9.99	1.26	51.2	1.38	30.0	.70	42.4	.30	52.8	.21	50.4	8.0	74.1	2.02
1	10.0 –19.99	.60	60.0	1.72	28.0	.68	56.0	0.34	52.0	0.13	60.0	4.6	66.3	18.1

[a] Excluding plant 2B.

percentage of fertility. Data for the fourth fertility class, 1.0%–9.99%, are somewhat skewed owing to inclusion of data for plant 2B. This class of five plants is characterized by a relatively low average frequency of laggards and nonfunctional pollen and a high average frequency of laggard-free sporocytes. Removal of the observations for plant 2B materially altered the average frequency of micronuclei at the quartet stage.

One plant (25A) was included in the 10.0–19.9% self-fertility class. Throughout the successive stages of division it had comparatively little meiotic irregularity, low pollen abortion, and high seed-set following self-pollination. The

in the I_1 generations from eight plants. Their S/Op percentages varied from 85.5 to 99.6. The population of I_1 plants was about equally divided between those that were depressed 5% or less and those exhibiting from 6 to 15% depression as expressed by the S/Op percentage.

Eight plants had from eleven to twenty plants in their I_1 progenies.The S/Op percentages were at least 95 for three, 85–94 for four, and 82.3 for one of the paired progenies.

In the group of progenies including thirty-one to forty plants, the average plant height of the I_1 generation of plant 25A was 71.9% that of its paired I_0 progeny. The S/Op percentages of the progenies from five plants were characterized by low losses in vigor (5% or less), while those of four additional plants were somewhat greater, these ranging from 6 to 10% reduction in plant height.

Analysis of the fifty-six plants as a whole indicates that 16.1% were self-sterile. Inbred progenies from thirteen plants, or 23.3%, were definitely inferior in vigor to their paired I_0 counterparts. An inbreeding depression of 10% or less occurred in progenies of thirty-four plants, these representing 60.7% of the total. The latter data did not take into consideration, however, the relatively small number of plants that made up the bulk of the progenies. These comparisons were also based solely upon plant height as an index of vigor as a measure of the effect of inbreeding.

DELAYED ALBINISM OR ETIOLATION.—Chlorophyll-deficient plants have appeared several times in progenies included in the breeding nurseries at Madison. This albinotic character differs from the more prevalent type generally expressed during the seedling stages of development in that the plants have the normal green coloration during juvenile stages and through the summer growth following establishment in the field during May. There was no evidence of abnormal coloration as plants entered the first winter in the field. During early spring certain plants differed from the usual stages of development in producing reddish, pale-yellow, or green and white foliage. The reddish and yellow-green pigmented plants appear to recover from this disturbance usually with the onset of warmer weather. White plants are sometimes completely devoid of normal or pale-green sectors, but others are characterized by some sectoring. With the arrival of warmer weather, the leaf tissue of the white plants deteriorates and collapses. In some cases death of the plant occurs, whereas others are again albinotic during the second spring following establishment. Representatives of this behavior are shown in figures 6 and 7.

This behavior essentially parallels that described by COLLINS (5), who estab lished a monofactorial explanation for the occurrence of albino barley plants grown at temperatures below 65° F.

Plants of two sets of twins exhibited this behavior, these including sets 25 and 26 isolated from a plant grown from a seed lot originally collected near Spooner, Wisconsin. One albino plant occurred in the I_1 generation from plant 25B, and there were two such plants in its paired I_0 progeny. No chlorophyll-deficient plants were noted in either progeny from plant A of this set. Three albinotic, and one pale yellow, plants occurred in the I_1 of 26A, whereas there was none in its I_0, and none in the I_0 or I_1 of 26B.

Discussion

Data presented here and in other publications by various workers are not yet sufficiently extensive to allow broad con-

clusions regarding this species. Some features appear, however, that will be discussed briefly.

It is fairly apparent that the various twin sets studied are not monozygotic. The morphological and cytological data are in good agreement with those derived from progeny tests to indicate that divergent germ plasm was involved in the conception of the members of a given twin set. The absence of twin combinations characterized by different chromosome numbers precludes the haploparthenogenetic formation of a second embryo from a cell other than the egg of a single developing female gametophyte. Apomictic forms of reproduction have not been observed by this writer, nor have they been reported to occur in the species (20). Certain other modes of origin alluded to earlier are not recognized, or have not been observed, to occur in smooth bromegrass. Thus, until the results of other studies are available concerning the origin of plural-embryo seeds, the mode of their formation must remain in doubt.

Based upon the information currently available, the cytology of this species is complex and indecisive. The meiotic behavior is known of the three plants purported to possess 28 somatic chromosomes. That reported by ELLIOTT and WILSIE (10) was quite regular; most of the chromosomes appeared as bivalents at diakinesis with a low frequency of multiple associations that were mostly disjoined by metaphase I. Nothing was stated regarding the following stages up to the quartet stage of development, but 87% of the quartets examined were free of micronuclei with a "high proportion of stainable pollen resulting." These observations are in rather sharp contrast to those made for twin set 24, of which the diakinesis figures were complex and in which from 7 to 9 bivalents were observed. There was a relatively high incidence of laggards throughout the subsequent division stages. Micronuclei were common, there being 0.38 and 0.77 such bodies per member cell of the quartets of plants A and B, respectively. Less than 30% of the quartets of both plants were free of micronuclei. No stainable pollen was found in the anthers of either plant. Some functional pollen was apparently produced during 1946, since some seed was set in the A plant following self-pollination. Whether these resulted from the presumed octoploid sporocytes has not yet been ascertained. It is difficult to account for the origin and behavior of plants of this set unless they are regarded as polyhaploid individuals that arose as a result of the failure of syngamy of the sperm and egg nuclei. Development of the embryos may have been stimulated as a result of fertilization of the polar fusion nucleus to form the primary endosperm nucleus. The low incidence of pairing in microsporocytes at diakinesis and the absence of stainable pollen all but preclude the consideration of these plants as representing the tetraploid members of a regular polyploid series such as those commonly encountered in the Gramineae.

The situation is equally confusing at the octoploid level. KNOBLOCH (16) reported comparatively regular divisions. As opposed to this, ELLIOTT and LOVE (9) concluded that, because of the cytological complexity observed in plants included in their study, there was some doubt as to whether inbreeding could be practiced toward the isolation of superior plant types to include in the synthesis of new and superior agronomic varieties. The observations on plants with low fertility recorded here would in part support the latter viewpoint. No plant examined

could be considered as being regular in its meiotic behavior, even though there was considerable variation in the amount of irregularity observed. Although the meiotic divisions in plants of the species are prevailingly irregular, the high polyploid structure apparently overcomes the deficiencies and excesses that result, or sufficient quantities of functional pollen and female gametophytes are produced to allow inbreeding through several generations.

The four decaploid plants studied were not greatly different from octoploid plants during the earlier stages of their meiotic divisions. They exhibited an intermediate or low frequency of micronuclei in the quartets and high percentages of stainable pollen.

The complexity of the prophase and diakinesis figures with their usual high frequency of multiple associations may be attributable to several processes. The synthesis of the species perhaps has followed a pattern somewhat similar to that postulated for certain other species of the genus by STEBBINS and several of his co-workers (25–30). Although the observations made upon the tetraploid biotype examined by ELLIOTT and WILSIE (10) indicate cytological structure approaching an autopolyploid, results reported here suggest an allopolyploid origin involving somewhat closely related species. Such an interpretation could account in part for the considerable homology that is expressed as multiple associations. This, coupled with an undetermined but perhaps large amount of segmental interchange, and some accompanying stickiness, would appear to be a logical supposition.

Substantial variations have been noted in growth forms occurring in breeding nurseries. Characters used to distinguish certain subspecific categories (12) as var. *villosus* Mert. and Koch (hairy lemmas); var. *pellitus* Beck (hairy lower leaves and sheaths); var. *divaricatus* Rohlena (small spikelets and pyramidal-triangular panicles); var. *pauciflorus* Rohlena (small spikelets with awned lemmas); var. *aristatus* Shur. (awns 2–4 mm. long); and var. *typicus* Beck (awnless and glabrous) would seem to suggest that at least a portion of the germ plasm entering into the synthesis of smooth bromegrass was of dissimilar nature. Segregation for such characters was apparent in the progenies grown.

That arguments such as those just presented might be deceiving is amply illustrated by the recent work of NORDENSKIOLD (21), who, through the repeated use of colchicine, synthesized hexaploid *Phleum* from the diploid *P. nodosum* L. Morphologically, the hexaploid was similar to the "wild type" of *Phleum pratense* L. Meiotically, however, the hexaploid was regular; the chromosomes appeared mostly as bivalents with a single quadrivalent present in approximately 20% of the sporocytes.

The genetic system that is operative in *B. inermis* is conducive to the maintenance of considerable heterozygosity. For the most part plants of smooth bromegrass are quite self-sterile, as is evident in the data presented. ADAMS (1) has shown that variations in cross-compatibility occur between unrelated plants also. This fact appears to be essentially analogous to the discrepancies that occurred between plants in the percentage of florets that formed seeds following self- and open-pollination. In some instances in the study reported upon here only a single seed was formed following bagging as compared with 400 or more following open-pollination. This ratio was much smaller in certain plants, being in the neighborhood of 1:10, 1:20, etc.

Similarly, progenies of certain members of twin sets were materially reduced in vigor following one generation of inbreeding, whereas those of the mates might be essentially of the same vigor class as the progeny derived following open-pollination. Likewise, some plants (25A, for example) that exhibited severe depression in the I_1 generation were less irregular meiotically than others not materially reduced. It would appear evident that genetic factors governing incompatibility, etc., constitute a significant role in the subsequent performance of plants of this species following inbreeding. The failures and successes cannot be attributed to cytogenetic disturbances alone.

From studies with alfalfa it has been suggested by TYSDAL and CRANDALL (**33**) that "clones relatively low in self-fertility tend to produce higher yielding progenies than clones relatively high in self-fertility" under Nebraska conditions. In the data concerning bromegrass included in this paper when the number of plants developed from seed following self-pollination was small, the ratio of the I_1 to the I_0 progeny might be unduly influenced by the development of exceptionally vigorous or very weak plants in the I_1 progeny. The data indicate, however, that these cases are approximately equal when the S/Op percentage, based upon four plants or less, falls below 100%. Thus, in smooth bromegrass it appears that self-fertility may not be directly related to exceptional vigor of the plants derived. This observation is based upon limited material, however, and should be recognized as subject to some question.

Data derived from the several aspects studied indicate that wide differences occur naturally within the species irrespective of whether vegetative or reproductive characteristics are considered. Approximately two-thirds of the plants examined are descendants from a foreign plant introduction from Manchuria. Some are the result of the inbreeding of plants developed from seed which was introduced. The plants in question exhibited approximately the same range of variability in the characters considered as other plants developed from seed taken from adventive colonies of *B. inermis* occurring in Wisconsin. It has been proposed that certain awned and pubescent forms of smooth bromegrass in the United States are attributable to introgressive hybridization involving *B. inermis* and *B. pumpellianus* (**8**). Although this interpretation might have considerable merit in western United States and Canada, the likelihood of interspecific hybridization having occurred extensively in the North Central States is open to some doubt. Some of the variations considered elsewhere suggest at least some similarity to certain subspecific categories included in HEGI's (**12**) treatment of the species. There is only meager evidence that *B. pumpellianus* occurred naturally in this region—aside from the extensive range and frequency with which it would have had to occur to modify effectively the natural diversity that exists in *B. inermis*. It is true that certain Cordilleran species do occur as disjunct outliers along the Great Lakes, particularly in northeastern Minnesota, the Keweenaw of Upper Michigan, and farther east adjoining the St. Lawrence River. Since there are no known specimens of *B. pumpellianus* taken from this midwestern region (except one presumably taken from cultivated material), in spite of the fact that the western Great Lakes areas mentioned have been rather thoroughly botanized, it would appear more reasonable to assume that in this

region the segregates in question perhaps represent introductions of European and Asiatic biotypes that are currently being confused with closely related western forms recently created as segregating populations as a result of hybridization of these species.

Summary

1. Twenty-eight sets of twin plants of *Bromus inermis* were successfully grown to maturity. Much of the original variation in vigor evident as seedlings had disappeared before the plants were established in the field, suggesting that the seedling differences in vigor were perhaps largely due to the relative positions of the embryos to the endosperm. All sets of twins differed in at least minor characters as adult plants.

2. Large differences occurred in seed-set following both self- and open-pollination. This was evident both between and within twin sets. The percentages of pollen stainable with IKI varied from 0 to 99.4, the differences being approximately as large within as between sets. Pollen of a given plant generally varied somewhat in size. Three major classes were found in the population examined, these having diameters approximating 50, 61–78, and 84–88 μ.

3. Variations were noted in the frequency of irregular meiotic divisions between and within sets. Generally there was a tendency for the amount of this irregularity to be inversely related to the extent of seed-set following both self- and cross-pollination.

4. The plants of the I_1 progeny from some plants were materially reduced in vigor when compared with those of the I_0 progeny. Those from other plants were not materially reduced in vigor following inbreeding, This relationship was evident both within and between sets. The data indicate also that low self-fertility may not be closely related to the occurrence of plants of superior vigor in the I_1 generation.

5. Comparisons within 28-, 56-, and 70-chromosome plants and their progenies indicate close similarity of behavior throughout gametogenesis irrespective of the degree of polyploidy. Seed-set was greater, however, in the higher polyploids, other behaviors being approximately equal.

6. It would appear evident that the twins were not monozygotic when considered with respect to the morphology and cytology of members of a given set. The behavior of the progenies following inbreeding and open-pollination was also in accord with this interpretation.

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STUDIES ON THE ORIGIN OF ETHYLENE FROM PLANT TISSUES[1]

WAYNE C. HALL

Introduction

In spite of the pronounced effects that ethylene apparently has upon the physiology of both individual plant organs and entire plants, its metabolic origin is still undetermined. In a recent review, BIALE (1) concluded that "we are dealing here with a substance which is physiologically active in minute quantities, but its activity cannot be traced to known mechanisms of substrate degradation." It is, however, generally agreed that only living or respiring tissue produces this active emanation. GANE (6) found that aerobically, but not anaerobically, growing yeast cultures produced a substance that prevented growth of etiolated pea seedlings. He (5) showed also that ripe apples in air give off emanations inhibitory to plant growth, but apples killed by freezing or kept in an atmosphere of nitrogen no longer produced gases effective in the pea test. HANSEN (11) confirmed this observation for pears and also supplied evidence for the relation of ethylene evolution to aerobic respiration. Carbon dioxide output from pears kept in an atmosphere of hydrogen or nitrogen remained unchanged, while ethylene production dropped sharply.

CROCKER (3) concluded from results obtained by the Boyce Thompson and other groups that all respiring plant tissue produces ethylene, although the amount produced varies greatly with the species of plants, the plant organ, and the age of the organ.

The present study was concerned with devising a suitable, rapid method to estimate quantitatively the amount of ethylene produced by plant tissues and the use of the method in *in vitro* studies to determine, if possible, the metabolic origin of this biologically active emanation.

The ethylene method

The most practical methods employed for the quantitative determination of ethylene have depended upon reaction of ethylene with either bromine or potassium permanganate. Most workers to date apparently have favored use of the bromination method or modifications of it (4, 5, 7, 12, 15, 16). As pointed out by PRATT *et al.* (16), although bromine is one of the most effective reagents for the removal of ethylene from a gas stream, it is also extremely difficult to manage owing to its highly volatile and noxious nature. Because of this, other reagents also were investigated for their adaptability in a quantitative method. The ability of ethylene to reduce dilute potassium permanganate solutions, after other potassium permanganate–reducing substances were removed from the gas stream with sodamide, was used successfully by NELSON (14) to determine quantitatively the ethylene production from apple fruits. NELSON's method, however, measured only the ethylene content of the tissue at a given time, as it necessitated killing the tissue. A combination elimi-

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nation test using dilute potassium permanganate, bromine water, and bromine–carbon tetrachloride solutions has been used previously by the author (9) to show qualitatively the origin of ethylene from ripening fruits.

Because of the large number of determinations necessary in the present study, after preliminary experimentation a modification of NELSON's $KMnO_4$ technique was selected as the most feasible for quantitative use. The absorption train used for the collection of ethylene, however, was patterned after the type described by PRATT *et al.* (16) and BIALE and SHEPHERD (2). The absorption of ethylene by means of the mercuric-perchlorate, ethylene-absorbing reagent as described by YOUNG *et al.* (19), followed by the liberation of ethylene into dilute permanganate, as well as direct absorption into dilute $KMnO_4$, has been investigated. In the first case an aliquot of freshly prepared permanganate was titrated with standardized ferrous ammonium sulfate. Another aliquot of this permanganate was placed in the receiving flask, and the ethylene from the mercuric-perchlorate complex was liberated under reduced pressure (14) into the receiving flask which was constantly shaken during the procedure. The $KMnO_4$ then was titrated as before with standardized ferrous ammonium sulfate. The difference between the titration values of the original $KMnO_4$ and of the $KMnO_4$ reduced by the ethylene liberated is converted to equivalent milliliters of ethylene at standard conditions (0° C. and 76 cm. Hg). In the second case the ethylene produced by the plant tissue was collected in the evacuation train by direct bubbling into an excess of standardized $KMnO_4$ during a 72–96-hour run, with the formation of ethylene glycol and manganese dioxide. Immediately at the end of the run the absorption towers were disconnected, and 25-ml. aliquots of the partially reduced permanganate were removed and filtered on a retentive filter. It was found that slight acidification with concentrated sulfuric acid greatly facilitated flocculation of the precipitate and the rate of filtering. The amount of acid used, however, should be carefully controlled, as an excess will in time destroy some of the permanganate color. The filtrate containing the unused $KMnO_4$ is then immediately titrated with standardized ferrous ammonium sulfate, and the titration value is subtracted from that of a $KMnO_4$ blank which was subjected to a 72–96-hour treatment in the evacuation train without tissue being present. The $KMnO_4$ blank was also acidified and filtered according to the above procedure. The difference between the two titration values is converted to equivalent milliliters of ethylene at standard conditions. The normality of the $KMnO_4$ used depends, of course, upon the amount and type of tissue being tested. In the present study 0.04 *N* $KMnO_4$ was found to be adequate in most cases.

Experimentation and results with the ethylene method

APPLES.—Mature, preclimacteric Red Delicious and Winesap apples were obtained as soon as possible following harvest and kept stored at 5° C. until ready for use. Prior to being placed in the evacuation chamber they were brought out of refrigeration and left at room temperature for 24 hours. Ethylene evolution from the Red Delicious variety was determined by means of the mercuric-perchlorate absorbing method, while Winesap fruit was used in the runs employing the direct $KMnO_4$ absorption technique. A comparison of the two techniques is summarized in figure 1. It is believed

that differences found between values determined by the two methods are attributable both to varietal difference and to the possibility that there was a secondary reaction taking place at room temperature between ethylene glycol and potassium permanganate over a prolonged period. However, for the range desired the two methods are for all practical purposes equally reliable, especially in showing relative trends. For this reason and because of the greater facility in using the direct $KMnO_4$ absorption method, it has been employed for the bulk of the measurements reported in this paper.

Computed on a weight basis, the average value for apples was 3.5 ml. of ethylene produced per kilogram at standard conditions. This falls within the range previously reported for apples and may be contrasted to the estimates of 0.12 mg/kg for McIntosh and 5 ml/kg for Permaine given by NELSON (**13**) and GANE (**4, 7**), respectively.

ORANGES.—Valencia oranges were obtained from the market, and the amount of ethylene produced during a 96-hour period was measured from intact oranges, oranges inoculated with the green mold *Penicillium digitatum*, and oranges cut into one-eighth segments. Cut oranges produced the most ethylene and were followed by the mold-inoculated oranges, while the intact fruit produced the least (fig. 2). It has been shown that the green mold, *P. digitatum*, is capable of producing ethylene when grown on artificial culture media. This probably explains at least part of the increased ethylene production by the inoculated fruit. There is also the probability that the mold secreted enzymes into the fruit which favored the hydrolysis of compounds that eventually served as substrates in ethylene production. GERHARDT and EZELL (**8**) noted a pronounced increase in total volatiles upon the onset of fungal attack but did not ascertain whether these substances had any effect upon the physiology of the host fruit. It has been shown also by WILLIAMSON (**18**) that shredded healthy leaves produce considerably more ethylene than uninjured healthy leaves. He concluded that this is a response to injury. It might also be possible that, in the case of the cut fruit, the greatly increased surface area exposed to oxygen and enzyme action is partly responsible for some of the increased ethylene output noted in this treatment.

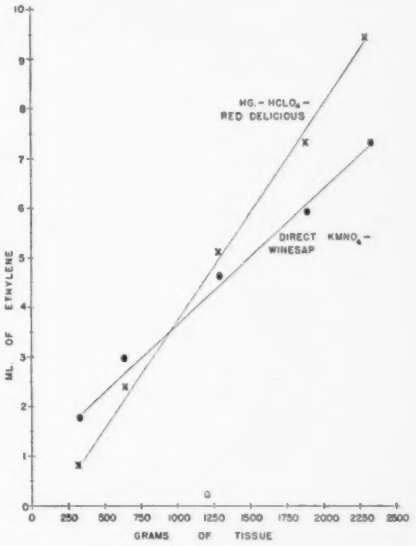
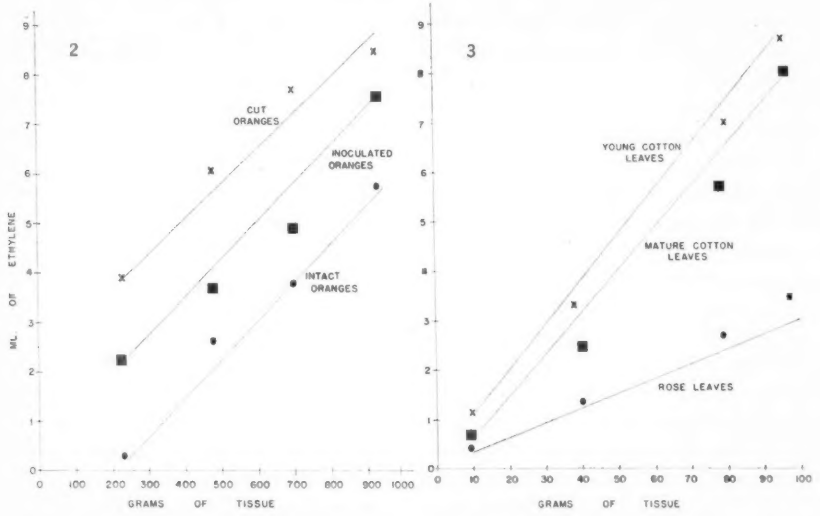


FIG. 1.—Comparison of ethylene production from Red Delicious apples using mercuric-perchlorate absorption method with ethylene production from Winesap apples using direct $KMnO_4$ absorption technique. Ethylene expressed as milliliters produced at standard conditions per weight of fruit.

A direct comparison of ethylene production by apples and oranges on the weight basis is somewhat misleading, as the average weight of the oranges used

was 120.5 gm. per fruit, whereas the average weight of the apples per fruit was 155 gm. This means that on the weight basis approximately one and one-third oranges were equivalent to one apple.

LEAVES.—Healthy mature leaves of *Rosa multiflora* were picked from plants free of disease, and their ethylene evolution measured (fig. 3). The amount of ethylene produced by young cotton leaves (preflowering plants) and mature cotton leaves (postflowering plants) of the variety Stoneville 2B TPSA was determined also (fig. 3). The petioles of the leaves were kept in water during the 96-hour determination. It was expected that rose leaves would produce more ethylene than cotton leaves. However, on a weight basis just the converse occurred. Even more unexpectedly, the young cotton leaves produced more ethylene on the weight basis than the mature leaves. Most authorities seem to be of the opinion that mature tissues produce more ethylene than young tissues. This variance between opinion and the observed results (fig. 3) is possibly explained by the fact that many more young leaves were required to equal the amount of mature leaves by weight, and hence the young leaves had a much higher total surface area producing the emanation.



FIGS. 2, 3.—Milliliters of ethylene produced per weight of tissue by (fig. 2) intact orange fruit, inoculated oranges, and cut orange fruit, and (fig. 3) rose leaves, young cotton leaves, and mature cotton leaves.

Studies on the origin of ethylene—substrate experiments

METHODS AND MATERIALS.—Most workers agree that only respiring plant tissue produces ethylene. This suggests that some normal plant constituent or complex of constituents serves as a substrate for possible enzymatic degradation during the course of respiration. Ripening fruits have been used for years as an excellent biological source of ethylene. Therefore, it was assumed that ripe apples contained both the enzyme sys-

tem and the substrate from which ethylene arises during respiration. To test this, ripe Red Delicious apples were pressed in a Carver hydraulic press at 1400 pounds per square inch. The juice was cleared with neutral lead acetate and sodium oxalate, filtered, and the clear filtrate utilized as the crude enzyme source for *in vitro* experiments with various substrates (table 1). A 1% concentration of all substrates tested was prepared from reagent grade chemicals and distilled water and autoclaved at 15 pounds for 15 minutes whenever the chemical structure of the compound was not likely to be altered by the sterilization treatment.

Five hundred milliliters of the substrate were then mixed with 100 ml. of the fresh apple-juice extract in a large sterile, 7-inch humid chamber, placed in the evacuation train, and ethylene production determined by the procedure outlined previously. One hundred milliliters of the apple-juice extract were also mixed with 500 ml. of sterile distilled water, and the amount of ethylene produced was determined according to the procedure. This value was subtracted from all determinations utilizing this enzyme source with other substrates given in the results.

Both BIALE and SHEPHERD (2) and WILLIAMSON (18) have observed that the common green mold, *Penicillium digitatum*, produces an active emanation when cultured on potato–dextrose agar. Apparently this fungus contains the necessary enzyme system (or systems) capable of liberating an active emanation by substrate degradation. Therefore, this organism was also utilized in the following manner as an enzyme source in the studies on ethylene origin. Cultures of *P. digitatum* were grown on potato–dextrose agar in Petri dishes until the mold covered the dishes and was sporulating heavily. The fungus mat and substrate from ten Petri dishes was transferred to a Waring Blendor and slurried with dilute acetone (free from permanganate-reducing substances) for 15 minutes. The entire mixture was then filtered on a Büchner funnel, and the filtrate cleared with neutral lead acetate and sodium oxalate. Fifty milliliters of the mold-acetone extract were incorporated into 500 ml. of the substrate to be tested, and ethylene evolution was determined according to the procedure previously described. The ethylene value for 50 ml. of the mold extract in 500 ml. of distilled water was also subtracted from all substrate determinations employing this crude enzyme source in accordance with the apple-juice enzyme-substrate values.

The substrates tested (table 1) by the potassium permanganate reduction method were checked also for ethylene production by the triple response of etiolated Alaska pea seedlings. The pea seedlings were cultured according to the technique of PRATT and BIALE (17) and were placed above 500 ml. of the tested substrate (2% concentration) plus either 50 ml. of the acetone-mold extract or 100 ml. of the apple-juice enzyme source contained in the bottom of large sealed desiccators. Observations were recorded after 72 hours. A relative scale from 0 to 5 is used in rating the activity of the substrates in producing permanganate-reducing substances and inhibition of the pea seedlings. The effect of increasing the concentration of some of the higher-yielding ethylene-producing substrates was quantitatively assayed, using both crude enzyme sources (figs. 4, 5). In like manner the effect of increasing the amount of the mold extract when substrate concentration was kept constant was also determined (fig. 6). The pro-

duction of ethylene by various sporulating fungi growing on different substrates in large 7-inch dishes was also recorded. A commercial pectin-hydrolase, Pectinol A, failed to produce any active emanation when tested upon a number of the organic acid, sugar, and pectin substrates. Negative results were obtained also using Taka-diastase and other commercial enzyme sources, as well as by conducting the experiments under anaerobic conditions.

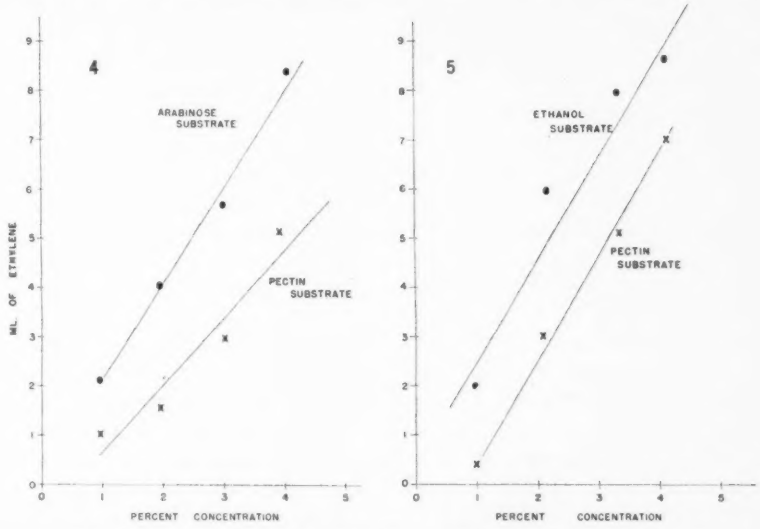
RESULTS.—Reference to table 1 indicates that a number of substrates can be utilized in the production of an active emanation under the influence of either the crude apple-juice enzyme or the mold-extract enzyme source. Combination of the potassium permanganate reduction test with the etiolated pea test

TABLE 1

IN VITRO STUDIES ON ETHYLENE PRODUCTION FROM VARIOUS SUBSTRATES UTILIZING EITHER APPLE JUICE OR MOLD EXTRACT AS ENZYME SOURCES. TEST RESPONSE REPRESENTED BY RELATIVE RATING FROM 0 TO 5

SUBSTRATE	$KMnO_4$ REDUCTION TEST		ETIOLATED PEA SEEDLING TEST	
	Apple juice	Mold extract	Apple juice	Mold extract
Carbohydrates:				
Hexoses:				
Glucose	1	1	Not run	0
Fructose	2	4	Not run	2
Galactose	1	4	Not run	3
Mannose	Not run	1	Not run	2
Pentoses:				
Arabinose	5	5	5	5
Rhamnose	Not run	2	Not run	2
Xylose	Not run	1	Not run	2
Disaccharides:				
Sucrose	1	1	1	1
Maltose	2	4	Not run	2
Polysaccharides:				
Starch	0	0	0	0
Organic acids:				
Acetic	Not run	2	Not run	2
Citric	0	2	Not run	2
Succinic	1	Not run	Not run	2
Malic	1	1	Not run	1
Fumaric	2	1	Not run	2
Pyruvic	4	3	Not run	1
Galacturonic	0	2	Not run	0
Maleic	0	0	0	Not run
Oxalic	Not run	2	Not run	1
Pectic	3	4	2	Not run
Alcohols:				
Methanol	Not run	4	Not run	4
Ethanol	5	5	5	5
Glycerol	Not run	0	Not run	0
Miscellaneous:				
Pectin	5	3	3	2
Agar	0	0	Not run	0
Gelatin	0	0	Not run	0

gives fairly conclusive evidence that the emanation is ethylene and some insight as to the relative activity of the substrates in their ability to produce this active gas. When either enzyme source was boiled in a water bath for 1 hour and then tested with the various substrates, they were found to be inactive in the pea test. Furthermore, 50 ml. of the mold-extract crude enzyme source on ethylene output from a uniform concentration of arabinose was reflected also in an approximate straight-line relationship (fig. 6). This demonstrates further that both the substrate and the enzyme source are intimately associated with the production of the active emanation. It was noted (table 1) that the hexoses appear



FIGS. 4, 5.—Relationship between ethylene production and increasing concentration of substrates when utilizing uniform amounts of (fig. 4) crude apple-juice enzyme source and (fig. 5) crude acetone mold-extract enzyme source.

acetone extract enzyme or 100 ml. of the apple-juice enzyme incorporated into 500 ml. of sterile distilled water gave only slight or negative inhibition in the pea test. This suggests that ethylene arises *in vivo* from enzymatic degradation of active substrates.

Approximately a linear relationship existed between the concentration of the more potent substrates of arabinose, pectin, and ethanol and their ethylene production (figs. 4, 5) when utilizing a constant amount of either enzyme source. The effect of increasing the amount of

to be somewhat better substrates for ethylene liberation in the case of the mold-extract enzyme, whereas the pentose substrates appear to be more active for the apple-juice enzyme source. In the *in vitro* experiments, ethanol, followed by arabinose, was the most potent of the substrates tested in producing ethylene (figs. 4, 5).

In vivo experiments with various fungi revealed that *Rhizopus nigricans* or *Penicillium italicum* failed to produce ethylene on any cultural media tested, although both are known to be potent pec-

tin hydrolyzers (table 2). *P. digitatum*, however, produced high amounts of ethylene when growing on a number of substrates (table 2). When l-arabinose was substituted for dextrose in the culture medium, growth of the mold was greatly reduced, but, in spite of the poor growth, ethylene production was not drastically impaired. This indicates that l-arabinose apparently is an excellent ethylene substrate source for this organism, even though it supports only scanty growth. BIALE and SHEPHERD (2) suggested that *P. digitatum* produced ethylene from dextrose. This is confirmed by the data (table 2) and indicates further that other fermentable substances also are capable of serving as potential ethylene-yielding substrates for this organism.

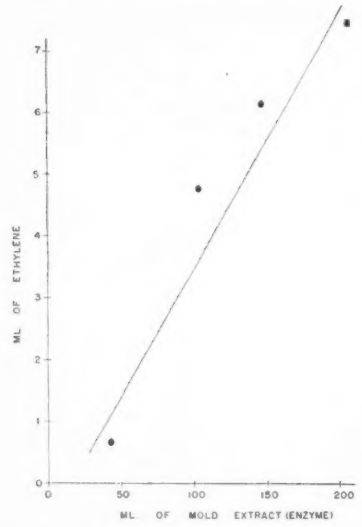


FIG. 6.—Relationship between ethylene production and increasing amounts of crude acetone mold-extract enzyme source when utilizing uniform 1% concentration of arabinose.

Discussion

The results obtained with the permanganate reduction method indicate that it is suitable for intensive quantitative studies on ethylene if the necessary precautions are observed. Limitations attending use of this method are largely overruled in favor of its simplicity and rapidity, particularly when accurate absolute values are not the primary consideration. Further screening by use of the etiolated Alaska pea seedling test enhances the reliability of the results.

In vitro experiments showed that ethylene may arise from a rather wide variety of substrates subjected to the attack of either the crude mold-extract or the apple-juice enzyme source. At present it appears that the only necessary requirement for ethylene evolution is that

TABLE 2

PRODUCTION OF ETHYLENE BY FUNGI GROWING ON VARIOUS SUBSTRATES IN 7-INCH DISHES

Organism	Substrate	Etiolated pea test	Ethylene produced (ml.)	Remarks
Rhizopus nigricans	Potato dextrose agar	Negative inhibition	None	Heavy excellent growth and fruiting
Penicillium italicum	Potato dextrose agar	Negative inhibition	None	Good growth and fruiting
P. digitatum	Potato dextrose agar	Positive inhibition	10.1	Heavy growth and fruiting
P. digitatum	Dextrose agar	Positive inhibition	9.8	Good growth and fruiting
P. digitatum	Arabinose agar	Positive inhibition	8.5	Poor, patchy growth, scanty fruiting

the substrate be a fermentable substance and that the reaction take place under aerobic conditions. Undoubtedly no single substrate yields ethylene directly, but it perhaps originates through a complex series of intermediates catalyzed by the necessary enzyme systems. It is suggested that this is paralleled in living plant tissues by a comparable respiratory mechanism complex eventually resulting in the liberation of ethylene. Evidence for *in vivo* liberation was shown in the substrate culture experiments in which the green mold, *Penicillium digitatum*, produced ethylene from either dextrose or arabinose agar media. Since agar, alone, was found to be inactive as an ethylene substrate, it is evident that the sugars were the original ethylene-yielding substrates.

In ripening fruits apparently ethylene may paradoxically function as an autocatalyst, accelerating its own production. It is interesting to speculate in light of the observed results that ethylene, in ripening fruits, first originates from degradation of intracellular substrates such as the simple sugars or organic acids. This in turn catalyzes the hydrolysis of the complex, insoluble, intracellular and cell-wall constituents, as the pectin compounds and the hemicelluloses, to soluble, ethylene-precursor substrates which are degraded during the respiratory mechanism of the climacteric. During this latter stage the peak of ethylene production occurs. Partial substantiation for this proposed sequence is shown by the fact that pectin is an excellent ethylene-precursor substrate, and, of its hydrolytic products, arabinose and methyl alcohol are even more potent sources (table 1). HANSEN (11) has noted that the rate of soluble pectin formation in pears paralleled that of ethylene production. HANSEN (10) studied also the effect of ethylene on chemical changes in pears and observed that an increased rate of starch hydrolysis, an increased sugar content, and more rapid transformation of propectin to pectin occurred in fruits of early maturity. The response to applied ethylene was identical with the changes taking place normally in more mature fruits. The length of time that individual compounds were affected by ethylene was shortest with sugars and longest with the pectic substances. Of special interest in conjunction with the present work was HANSEN's observation that respiration and pectins ceased to respond to ethylene at about the same time.

Of the substrates measured, ethanol unexpectedly produced the highest amounts of ethylene. Although sufficient evidence is not yet available to warrant a final conclusion, this might indicate that the various potential ethylene-yielding substrates are eventually reduced to alcohol, which represents the terminal substrate from which ethylene arises. The common method for the preparation of ethylene in the laboratory by treating alcohol with sulfuric acid is well known. Because sulfuric acid is regenerated in the process, it is generally regarded as a catalyst, by some authorities, although others state that its primary role is that of a dehydrating agent. The possibility that living tissue, employing enzymatic catalysts, produces ethylene in a similar manner should not be overlooked and definitely merits further investigation.

Summary

1. A relatively rapid, quantitative method for estimating the production of ethylene from plant tissues is reported. The method is a modification of NELSON's $KMnO_4$ reduction technique and consists essentially of a ferrous ammoni-

um sulfate–$KMnO_4$ titration procedure involving the collection of ethylene in an excess of $KMnO_4$ with the formation of ethylene glycol and manganese dioxide. The $KMnO_4$ absorbing solution is then cleared of these constituents, and the unused $KMnO_4$ titrated at the end of the run. The titration value is converted to equivalent milliliters of ethylene at standard conditions.

2. The ethylene value for apple fruits determined by the $KMnO_4$ reduction method falls within the published range, obtained by various workers using other methods, for this type of tissue. Intact Valencia oranges produced considerably less ethylene than when the fruit was cut into segments or inoculated with *Penicillium digitatum*. Leaves of rose and cotton also produced measurable amounts of ethylene. Young cotton leaves were found to evolve more ethylene than an equal weight of mature cotton leaves.

3. *In vitro* studies employing various common compounds from plants and a crude enzyme source obtained from either apple juice or the green mold, *Penicillium digitatum*, revealed that ethylene does arise by enzymatic degradation of a number of active substrates during respiration. A suggested mode of its origin in living tissue has been proposed and discussed.

The writer gratefully acknowledges the technical assistance of Mr. HARRY C. LANE in conducting the ethylene determinations as well as the helpful suggestions of his colleagues in the Department of Plant Physiology and Pathology during the preparation of this manuscript.

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MORPHOLOGICAL AND ANATOMICAL EFFECTS OF 2,4-D ON YOUNG CORN PLANTS

ROBERT W. HOSHAW[1] AND ARTHUR T. GUARD

Introduction

Discovery of the weed-killing properties of 2,4-dichlorophenoxyacetic acid (2,4-D) has proved to be one of the most important developments in agriculture since the introduction of hybrid corn. In general, interest has centered around the killing action of 2,4-D, with little regard to the causes for this action. Anatomical studies of plants subjected to 2,4-D treatments may throw some light on the fundamental action of such a growth-regulator. Several studies of this nature have been made on dicotyledonous plants, while few investigations (**10**, **18**) have involved the anatomical response of monocotyledonous plants to 2,4-D.

Variations in response to 2,4-D are quite noticeable among various lines of corn. LEE (**14**) concluded that growing conditions rather than the size of the plant affected the tolerance of corn to 2,4-D. BUCKLEY (**6**) noted that corn plants treated when 8–12 inches high showed noticeable bending within 24 hours. Later the brace roots of these plants grew together, forming thickened disks. HOLDEN *et al.* (**13**) treated sixteen hybrid corn varieties with a 2,4-D ester when the plants were 22–26 inches high and found proliferations of brace roots, bending, breaking of stalks, and much reduced yields. BUCHHOLTZ (**5**) used a wide range of plant heights and observed the most pronounced bending at the 24- and 36-inch stages with a marked reduction in yield when treated at the 24-inch stage. The effect of 2,4-D on the root development of young corn plants has been studied (**18**). In the present investigation the first internode, coleoptile, and lower young internodes of young corn plants were studied morphologically and anatomically in relation to 2,4-D treatment, and the observations are herein reported.

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Material and methods

The corn single hybrid, Wf9 × Hy, was grown in the greenhouse during the late winter and early spring of 1949 and 1950. The plants or soil was sprayed with an aqueous solution of the triethanolamine salt of 2,4-D which contained 39% of 2,4-dichlorophenoxyacetic acid equivalent. The spray was prepared from the commercial compound "Dow-40," supplied by the Dow Chemical Company. It was applied with a Hudson hand sprayer with an adjustable nozzle. A computed amount of spray was directed on each pot, taking into consideration its soil area. The plants were grown in 6-inch clay pots with six plants per pot

for the pre-emergence treatment and two plants per pot for the post-emergence treatment.

The plants were subjected to pre-emergence and post-emergence treatments with concentrations of 3000 and 5000 p.p.m. of 2,4-D. Plants which received the post-emergence treatment were 8–12 inches in height; the spray was directed at all parts of the plant, with most of the spray being intercepted by the leaves. In the pre-emergence treatment the soil was uniformly wetted with the spray material 2 days after planting, and no surface watering was done after this application.

Material collected from plants subjected to pre-emergence treatment consisted of the first internode and the coleoptile with its inclosed leaves; it was collected 32, 48, 96, and 240 hours after the plants emerged. From the plants given the post-emergence treatment, the first internode and the other of the lower internodes, including the growing point, were collected 4, 8, 12, and 21 days after the spray application. All material collected periodically for anatomical study was preserved in formalin-alcohol-acetic acid, imbedded by the butyl alcohol–paraffin method, and sectioned at 15μ. Safranin was the only stain used in the preparation of permanent slides.

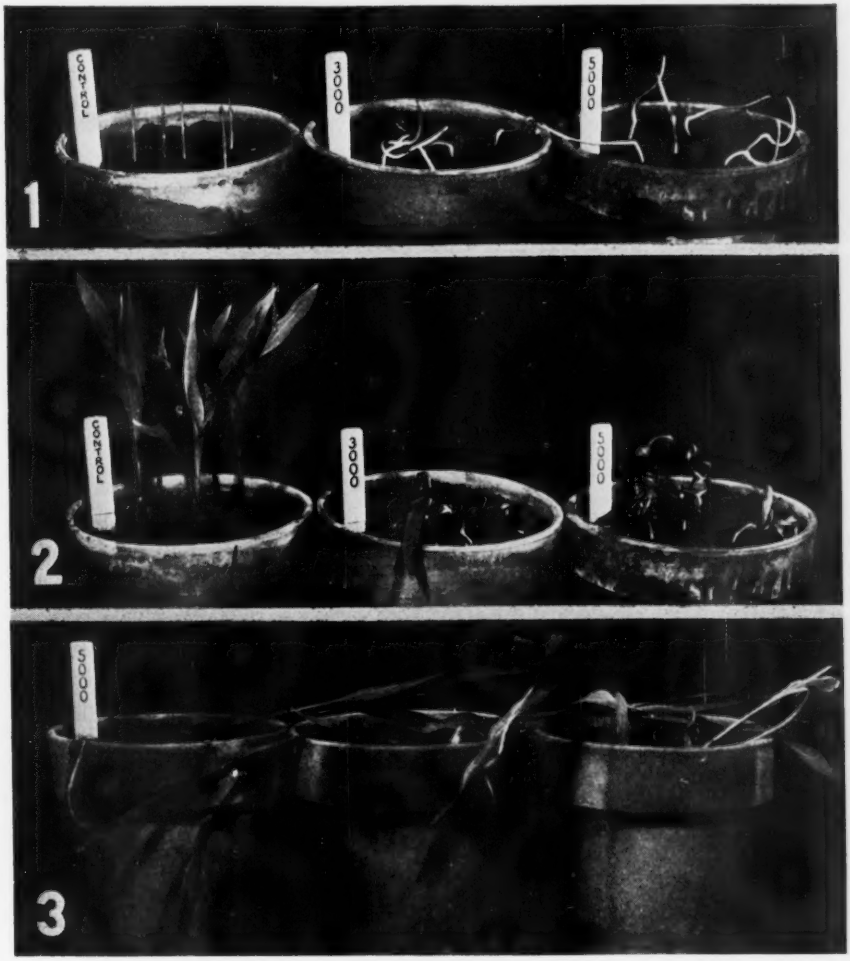
Observations

MORPHOLOGY.—As soon as the young seedlings emerged following the pre-emergence treatment, extreme bending and twisting of the coleoptiles were evident. Very little difference in morphological response was observed between the 3000-p.p.m. and the 5000-p.p.m. series (fig. 1). The coleoptiles twisted considerably and elongated far beyond the extent of the first leaves, thus leaving the tip region of the coleoptiles empty. The first internode of each plant was also excessively elongated and extended some distance above the soil level. At the same time, the first leaves of the untreated plants were emerging normally from the coleoptiles. At 72 hours the leaves of both groups of treated plants were breaking through the sides of the coleoptiles. This expansion of the leaves resulted in greatly distorted and twisted plants (fig. 2). Although the treated plants were extremely malformed, all were still alive at the end of the 10-day collection period. By 42 days after emergence, about 50% of the treated plants remained alive and appeared likely to grow to maturity. The twisting and bending were mostly overcome, and the living plants were growing upright, although somewhat stunted in appearance. Certain of the roots of these plants were twisted, and others were thickened and fasciated.

Plants in the post-emergence series responded similarly to the 3000-p.p.m. and the 5000-p.p.m. treatments. Within 24 hours after application, bending had occurred at the lower nodes. This bending intensified, and by the end of 12 days after application most of the shoots were in a horizontal position (fig. 3). By the end of 5 weeks, however, the treated plants had recovered or partially recovered from the horizontal position but were markedly stunted. Their average height was considerably less, and their leaf blades were much narrower, than those of untreated plants. Large masses of root tissue occurred just above the soil surface, and some of the fasciated roots failed to penetrate the soil.

ANATOMY.—Anatomical examination of plants which received the pre-emergence type of treatment indicated that the response to 2,4-D is rapid in the case of the two concentrations applied. By 32

hours after the plants emerged, a few cells were undergoing division, and these ultimately resulted in proliferated areas. The increase in meristematic activity preceded the formation of adventitious roots that were either normal in appearance or much enlarged and flattened. The formation of structures other than adventitious roots was not observed. Both groups of treated plants had much elongated coleoptiles which extended considerably beyond the inclosed leaves. This extreme elongation resulted from the increased length of the coleoptile



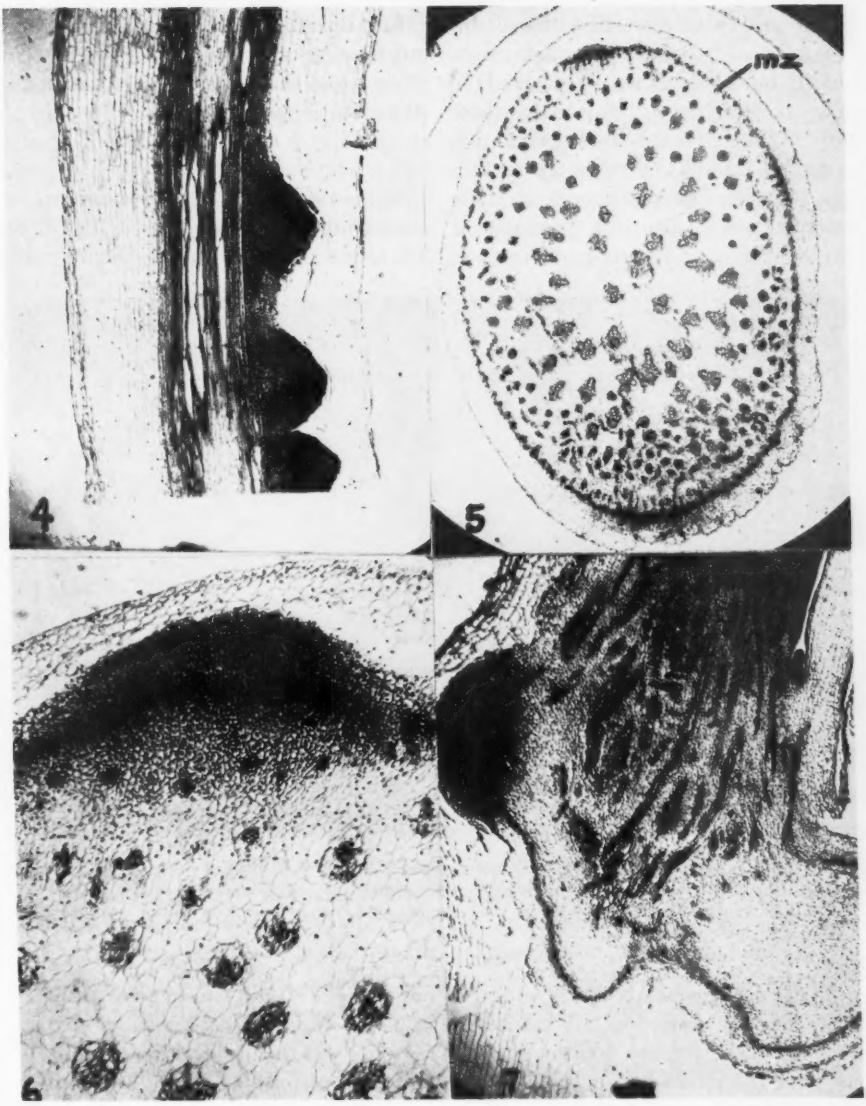
FIGS. 1–3.—Fig. 1, 48 hours, and fig. 2, 144 hours, after corn plants emerged following pre-emergence treatment with triethanolamine salt of 2,4-D. Characteristic bending and twisting of coleoptiles are evident at both 3000-p.p.m. and 5000-p.p.m. concentrations. Fig. 2 shows manner in which leaves emerge from coleoptiles. Fig. 3, condition of plants 12 days after being subjected to 5000-p.p.m. post-emergence application of 2,4-D. Bending occurs at lower nodes, but plants eventually return to an upright position.

cells and not from an increased number of cells in the coleoptile itself.

Active cells were easily identified in the first internode of treated plants. This first internode has been described by HAYWARD (12) as being both rootlike and stemlike in structure. Well-developed adventitious root primordia were noted in material of the first internode examined 96 hours after the plants emerged (fig. 4). It was the pericyclic cells that were in a meristematic condition, and remnants of the endodermis were pushed outward by the enlarging root primordia. It was concluded that the endodermis was unresponsive to 2,4-D. Likewise, the epidermis and cells of the cortex were not visibly responsive to the 2,4-D application. The inner tissues of the stele had matured rapidly and also failed to return to a meristematic condition. Observations made 10 days after emergence of the plants indicated that, in addition to well-developed adventitious roots, a ring of proliferated tissue, several layers in thickness, surrounded most of the outer periphery of the stele in treated plants. This tissue consisted of small isodiametric cells which lacked differentiation.

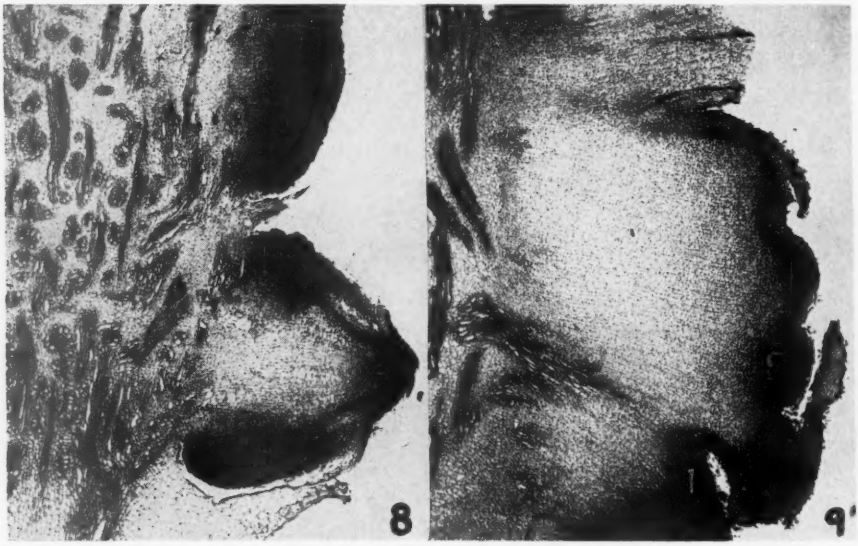
The general nature of the anatomical response to post-emergence treatment was similar to that obtained with pre-emergence treatment, but a lack of activity was evident in the first internode. Major response to treatment was evidenced by increased activity in the meristematic zone of the lower internodes other than the first one. This meristematic zone is a band of tissue which forms the outer limit of the stele and is in a position usually occupied by a pericyclic region. Adventitious roots originated in the young internodes from the proliferation of the tissue in the meristematic zone. Proliferation in the meristematic zone was clearly visible in plant material 21 days after it had received either concentration of the post-emergence spray. Early proliferation proceeded in an irregular manner. The parenchyma cells immediately inside the meristematic zone and the vascular units of the zone became active during the formation of proliferated areas. The activity appeared to occur inward at a much more rapid rate. The outer limit of the active area was particularly well defined, as a root primordium began to form from the proliferated area. In older proliferated areas it was possible to see three distinct regions (figs. 5, 6). Each region could be easily characterized by the nature of the cells within it. In all three regions rows of isodiametric or slightly elongated cells were formed. The outer region consisted of moderately meristematic cells which were roundish in appearance. In the narrow middle region were small, flat cells that were extremely meristematic. The cells of the inner region were the largest and the least meristematic, and the entire region appeared to be larger than the middle or outer regions. Proliferation moved inward from the inner region, but certain of the vascular cells did not immediately become meristematic. A comparison of a young proliferated area with an older proliferated area showed that the three regions were the histogens of a corn root tip.

A striking response of all treated plants was the manner in which roots developed as a result of the 2,4-D stimulus. Root abnormalities were more noticeable, however, in plants subjected to post-emergence treatment. The two concentrations of 2,4-D used in the pre-emergence treatment fostered the formation of large, flattened masses of root-forming tissue (fig. 7). The additional tissue of the root consisted of small isodia-



FIGS. 4–7.—Sections showing anatomical responses to 2,4-D treatment. Fig. 4, longitudinal section of first internode, showing adventitious roots arising from pericycle 96 hours after plant emerged following pre-emergence treatment. Fig. 5, cross section of a lower internode 21 days after treatment with 3000 p.p.m. 2,4-D. Note early stage of proliferation in meristematic zone (*mz*). Fig. 6, meristematic zone at periphery of stele, showing three distinct regions of activity in a lower internode of plant 21 days after treatment with 5000 p.p.m. 2,4-D. Fig. 7, longitudinal section from a plant 10 days after emergence in 5000-p.p.m. treated group indicating a normal root primordium and a large flattened root with additional undifferentiated tissue.

metric cells which were without differentiation. Eight days after being subjected to both concentrations of 2,4-D in the post-emergence treatment, these plants had numerous root primordia, originating laterally along more mature roots (fig. 8). The outer cells of these primordia were loose and produced a slimy condition of the roots. These primordia did not appear to enlarge and frequently became flattened. By 21 days after the post-emergence treatment, extreme root fasciation had occurred (fig. 9). Numerous root primordia originated from the massive areas of proliferated tissue, and it appeared that certain of these primordia would elongate and become single roots. The tissue which formed the massive areas consisted of small isodiametric or slightly elongated cells that were without differentiation. The outer cells of these large proliferated areas were also loose and produced a slimy condition near the basal portion of the stem of each plant.



FIGS. 8, 9.—Longitudinal sections showing root abnormalities. Fig. 8, from plant given 3000-p.p.m. post-emergence treatment. Note occurrence of small root primordia with their loose outer cells which promote slimy condition of root. Fig. 9 shows extreme fasciation of root-forming tissue 21 days after post-emergence treatment with 5000 p.p.m. 2,4-D.

Discussion

The use of either pre-emergence or post-emergence spray applications of the triethanolamine salt of 2,4-D resulted in morphological and anatomical response in young corn plants. The most striking response was the proliferation and fasciation of root-forming tissue. This tissue consisted chiefly of numerous undifferentiated cells. Less noticeable proliferation occurred near the periphery of the stele in both the first internode and the young stem internodes. From this meristematic activity adventitious roots originated.

The formation of adventitious roots

from the first internode of treated plants was common during the early stage of development of this axis. According to AVERY (2), adventitious roots may arise at any level between the cotyledonary and coleoptilar nodes. However, in the many sections of untreated material which were studied from the pre-emergence group, no roots were visible in the first internode; in a few instances poorly developed adventitious roots originated from the pericycle of the first internode in plants of the post-emergence group. It is concluded that the numerous root primordia which originated in the first internode of treated plants resulted as a response to the 2,4-D application.

It was during the early stages of plant development that the first internode and 2,4-D were closely related. Response of this structure to both types of 2,4-D application resulted in activity at the periphery of the stele. This proliferation was confined to the pericycle and did not appear to interfere with normal development of the vascular system in the first internode. Proliferation also occurred in the meristematic zone of the lower internodes. This activity has been previously reported (18, 20). Such activity does not appear to disturb the normal development of other tissues of the young stem.

The most perceptible response was the fasciation of root-forming tissues which was either slight or evidenced by large masses of undifferentiated tissue. This lack of organization of the root system indirectly contributed to the stunted condition of the plants. In certain cases individual roots formed from the large masses of tissue; however, in other instances only collars of tissue formed, and they usually failed to penetrate the soil.

It has been reported (15, 19) that rather extreme responses may be obtained between various inbreds and hybrids. Such differences may be related to the manner of plant development—particularly to the speed of growth. LEE (14) pointed out that the tolerance of corn to 2,4-D does not depend on plant size but on the rate of growth at the time of treatment. All plants subjected to the post-emergence treatment were growing rapidly and responded to the spray applications within 24 hours. Response of the pre-emergence group occurred as soon as the plants reached the soil surface and came into contact with the 2,4-D material. These plants were then markedly affected all through their period of rapid growth. The plants in the pre-emergence group which survived the first 4 or 5 weeks appeared as though they might grow to maturity, although they were somewhat stunted in their development. Likewise, the treated plants of the post-emergence group were stunted, but it seemed as though they would grow to maturity.

The nature of the two methods of application makes a comparison of the morphological responses difficult. However, bending was a common response to both types of treatment, and in both groups the plants were stunted as they developed toward maturity. Two different types of 2,4-D treatment resulted in similar anatomical response. The tissue at the periphery of the stele was the center of meristematic activity in response to both types of treatment. Adventitious root initiation was a usual response. Tissues other than those at the outer limit of the stele were unresponsive visibly, even at the highest concentration of 2,4-D used. Plants of the pre-emergence group were subjected to the effect of 2,4-D at a much earlier stage in their development, and this should account for the greater activity of the pericyclic tissue in the first internode. Thus it appears in corn

that young tissues are much more sensitive to the 2,4-D stimulus than are older tissues.

The authors do not know of any investigation in which the anatomical response to a pre-emergence treatment of a growth-regulator has been studied. It would not be expected that the use of a pre-emergence 2,4-D treatment would induce significantly different anatomical response from that produced by the more usual foliar type of application. In the corn plant it was found that the anatomical response to the pre-emergence treatment involved the activity of the same tissues as were responsive in the post-emergence treatment. In the case of the pre-emergence treatment the tissues were much younger when they were subjected to the 2,4-D stimulus. It was found that such immature tissues responded readily to 2,4-D. Increased response to the pre-emergence treatment could be expected under field conditions, particularly if rain occurred soon after treatment and before the plants emerged. It has been reported (**21**) that all corn seedlings died within 10 days following a simulated rain when they had been previously subjected to a pre-emergence 2,4-D treatment. ANDERSON and WOLF (**1**) reported that a pre-emergence treatment 8 days after planting resulted in very little plant injury.

The response of monocotyledonous plants to growth-regulators has previously been compared with that of dicotyledonous plants (**9, 10, 18**). In both groups anatomical response occurs chiefly at the outer limits of the stele. For example, in the bean plant BEAL (**4**) found that in the second internode the endodermis, cambium, phloem, and ray parenchyma were activated with the formation of adventitious roots. In corn it was the pericycle of the first internode that responded with the formation of adventitious roots. Other higher internodes of corn showed activity in the meristematic zone. The proliferated tissue of both monocotyledons and dicotyledons was potentially root-forming tissue. The dicotyledonous bean plant responds to much lower concentrations of 2,4-D than does the monocotyledonous corn plant. This may be attributed to the differences in growth pattern and position of the meristems of these two groups of plants. It also has been suggested (**18**) that translocation of the 2,4-D stimulus is limited in the corn plant as compared with dicotyledonous plants. This suggestion is supported by the results of MITCHELL *et al.* (**16**), which showed that oat and corn plants accumulated appreciably less of the labeled growth-regulator 2-iodo^{131}-3-nitrobenzoic acid (INBA) than did bean plants. The lack of inhibition to oat and corn plants was attributed to the manner in which INBA reacted with plant constituents. In an experiment with the same growth-regulator WOOD *et al.* (**25**) found that radioactive INBA accumulated in the bud and hypocotyl of bean and in the second leaf of barley. In a recent preliminary investigation conducted by the senior author it was found that the translocation of 2,4-D, although not rapid, was quite noticeable in corn seedlings within 48 hours after application, as evidenced by morphological responses. This response is slow when compared with the immediate response of the bean plant to 2,4-D.

It is difficult to evaluate the relative responsiveness of corn to growth-regulators such as 2,4-D. In comparing the observations just described with those reported by other authors (**3, 4, 7, 8, 11, 17, 22, 23, 24**), it is apparent that corn is very tolerant of 2,4-D applications.

However, this tolerance is not complete, and in certain instances plants were killed during the present investigation. Thus a concentration of 5000 p.p.m. of 2,4-D in the quantity applied was toxic to the young corn plants. Such a high concentration should not be recommended for field use. The exact reason for the extreme tolerance of corn to 2,4-D remains obscure. It is probable that many factors are involved, such as position of the growing point, speed of translocation, number of meristems, and toxicity to plant constituents.

The apical meristem of corn is protected from the direct effect of 2,4-D spray applications. If the plants are young at the time of treatment, the growing point may still be below the soil level. This is in contrast to many plants which have their apical meristems fully exposed. The translocation of 2,4-D in corn, as evidenced by the telemorphic effects, is slow. Thus, even though 2,4-D is systemic in its action, the rate of movement of the material is not such that the entire plant is affected immediately after application. The lack of a cambium in the first internode and in the vascular bundles of the stem means that there is one less meristematic tissue in which proliferation might occur. The effectiveness of 2,4-D may be explained at some future date by the toxic effects it has on various plant constituents; it is intimated that the effect of 2,4-D on both living and nonliving constituents should be determined if an understanding of its action is to be obtained.

Summary

1. An investigation to determine some of the morphological and anatomical responses of a corn single hybrid, Wf9 × Hy, to the triethanolamine salt of 2,4-D (2,4-dichlorophenoxyacetic acid) has been described.

2. All plants were grown in the greenhouse during the winter and early spring and were subjected to either pre-emergence or post-emergence treatments at concentrations of 3000 p.p.m. or 5000 p.p.m. of 2,4-D. The anatomical study was made from material collected and preserved periodically up to a total of 21 days.

3. Both pre-emergence and post-emergence spray applications resulted in morphological and anatomical response in young corn plants. The most striking response was the proliferation and fasciation of root-forming tissue. Large masses of undifferentiated cells were observed. Less noticeable proliferation occurred near the periphery of the stele in both the first internode and the young stem internodes. Adventitious roots originated from this proliferated tissue.

4. Extreme morphological response to the pre-emergence treatment resulted in plants with much elongated coleoptiles and first internodes. Most plants also showed severe bending and twisting. The plants subjected to the post-emergence treatment exhibited extreme bending. However, the recovery of all plants subsequent to treatment was remarkable.

5. Observations of young stem internodes made 21 days after either 3000-p.p.m. or 5000-p.p.m. pre-emergence or post-emergence treatment showed proliferation in the meristematic zone. There were three regions visible in the proliferated area, and these corresponded to the three histogens found in a corn root tip.

6. Root fasciation was extreme, particularly in plants examined 21 days after post-emergence treatment. Large masses of tissue were externally visible. This tis-

sue consisted chiefly of small isodiametric or slightly elongated cells.

7. Anatomical examination indicated that tissues other than the pericycle and meristematic zone were unresponsive to the 2,4-D treatments used.

8. The tolerance of corn to 2,4-D is discussed. It is suggested that factors such as position of growing point, rate of translocation, number of meristems, and toxicity to plant constituents may contribute to the limited response of the corn plant to 2,4-D.

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RESPONSE OF CERTAIN VARIETIES OF GRAPES TO PLANT GROWTH-REGULATORS

ROBERT J. WEAVER AND W. O. WILLIAMS

Introduction

When flower clusters of Black Corinth grapes were treated with beta-naphthoxypropionic acid, larger berries developed that ripened 2 weeks earlier than untreated controls. Those treated with 4-chlorophenoxyacetic acid produced many large berries containing hard, seedlike structures,[1] and clusters of Thompson Seedless treated with 4-chlorophenoxyacetic acid developed larger berries than did untreated clusters (3).

The experiments reported here were made to test more thoroughly the response of clusters of these varieties to the above-mentioned growth-regulators and to determine the response to spray applications to entire vines of Black Corinth and Thompson Seedless as compared with dipping of clusters. Other varieties of grapes were also tested with these and other growth-regulators.

Material and methods

An irrigated vineyard located about 1 mile west of Davis, California, was used. Mature vines of Black Corinth, Thompson Seedless, Sultana, White Corinth, and Black Monukka were cane-pruned, and Muscat of Alexandria and Hunisia were head-pruned (4). Fruits of the latter two varieties contain seeds; those of the others are seedless. All are varieties of *Vitis vinifera*.

Mean monthly temperatures during the experimental period, May to September, inclusive, were 64.9°, 69.1°, 76.4°, 74.7°, and 71.4° F., respectively. From June 30 through July 3, inclusive, daily maximum temperatures ranging from 109° to 113° F. resulted in some injury to foliage.

Aqueous solutions of the growth-regulators tested (table 1) were prepared by adding sufficient ammonia to dissolve the compounds and by adding Dreft, 0.2% by weight, as a wetting agent. Oil emulsions of compounds that would not dissolve in water were prepared by dissolving separately 1 gm. of each compound in 5 ml. of an oil-base carrier (3) and then diluting to 1000 ml. with water, except that 10 ml. of carrier were used in case of the naphthaleneacetamide. At the concentrations used, preparations of Dreft or of carrier alone resulted in no injury.

Clusters of flowers or fruit were treated by momentary immersion in a formulation of one of the growth-regulators. Entire vines were sprayed with a 3-gallon hand sprayer. The foliage and clusters were sprayed heavily.

Average weight of berries was used as an indication of size with Thompson Seedless. Some berries were removed from all clusters of a given lot. These were thoroughly mixed, and then duplicate lots of 200 berries were weighed.

Experimentation and results

Response of flower clusters of Black Corinth to various growth-regulators

The purpose was to observe the response of flower clusters to dipping in formulations of thirteen additional

[1] Almost all these hard structures in Black Corinth, hereafter referred to as "seeds," were hollow.

TABLE 1

DEGREE OF INJURY* RESULTING FROM GROWTH-REGULATORS APPLIED TO FLOWER CLUSTERS OF GRAPE VARIETIES BLACK CORINTH, MUSCAT OF ALEXANDRIA, OR HUNISIA. OBSERVATIONS MADE ON AUGUST 9, SEPTEMBER 19, OR JULY 17, RESPECTIVELY. AQUEOUS SOLUTIONS USED UNLESS OTHERWISE INDICATED.

Growth-regulator	Concentration of growth-regulator (p.p.m.)	Black Corinth	Muscat of Alexandria	Hunisia
Beta-naphthoxypropionic acid	200	+	+++	+++
	20	o	++	o
	2	o	o	o
Alpha-(2,4-dichlorophenoxy)-propionic acid	20	+++	+++	+++
	2	++	+++	+++
	0.2	o	o	o
2,4,5-Trichlorophenoxyacetic acid	50	.....	+++	+++
	5	.....	+++	+++
	0.5	.....	o	++
Alpha-(2,4,5-trichlorophenoxy)-propionic acid	50	+++	+++	+++
	5	+	+++	++
	0.5	o	o	o
Beta-(3-indole)-propionic acid	200	o	.....	.....
	20	o	.....	.....
4-Chloro-o-toloxyacetic acid	500	+++	.....	.....
	50	++	.....	.....
Maleic hydrazide†	1000	+	.....	.....
	100	o	.....	.....
O-isopropyl N-phenylcarbamate‡	500	o	.....	.....
	50	o	.....	.....
Naphthaleneacetamide‡	200	+	+++	++
	20	o	o	o
	2	o	o	o
Alpha-(4-chlorophenoxy)-propionic acid	50	++	++	+
	5	+	+	o
	0.5	o	o	o
4-Chlorophenoxyacetic acid	50	o	+	+
	5	o§	o	+
	0.5	o§	o	o
Alpha-(1-naphthoxy)-propionic acid	200	o§	.....	.....
	20	o	.....	.....
2,3,5-Triiodobenzoic acid	200	o	.....	.....
	20	o§	.....	.....
N-2-chlorophenylphthalamic acid†	500	o§	++	++
	50	o	o	o
N-1-naphthylphthalamic acid†	200	o§	+++	+
	20	o	o	o
	2	o	o	o
N-phenylphthalimide†	1000	+§	.....	.....
	100	o	.....	.....

* The symbols for degree of injury are as follows:
o = No injury.
+ = Slight injury. Usually a slight curvature of rachis occurred. Clusters recovered and developed normally, except that some berries failed to enlarge.
++ = Medium injury. Many berries failed to enlarge. Rachis usually enlarged, split, and callused. Tip of cluster was sometimes dead.
+++ = Severe injury. Cluster dead and dry, or if alive, less than 5% of berries had enlarged, and rachis was twisted and split.

† Compounds supplied by the Naugatuck Chemical Division, U.S. Rubber Company, Naugatuck, Connecticut. Maleic hydrazide was formulated as the water-soluble diethanolamine salt of 1,2-dihydropyridazine-3,6-dione.

‡ Oil emulsions used.

§ Large clusters and berries. Many berries contained hard seeds.

growth-regulators not previously tested on Black Corinth (table 1). For comparison, 4-chlorophenoxyacetic acid and beta-naphthoxypropionic acid were included (3). Clusters on ungirdled vines were treated on May 22, when most of the flowers were in bloom—about 90% of the calyptras had fallen. At that time the trunks of four vines were girdled, but their clusters were not treated. There were eight clusters per treatment, except that only six were used in case of the first three compounds listed in table 1.

As usually occurs with this variety, most untreated clusters on ungirdled vines dried without further development, although in some instances the clusters at harvest on August 9 were still alive with a few small berries. Normally, Black Corinth must be girdled in order to develop large, seedless berries. The compact clusters on girdled vines had enlarged berries typical of those developing after girdling.

The growth-regulators caused varying degrees of injury. Four days after treatment the rachis, its branches, and the pedicels of many treated clusters showed curvatures. The compounds which induced increased set and enlarged berries did not usually cause much injury.

Applications of beta-naphthoxypropionic acid did not increase the rate of maturation. Apparently, the set of berries was somewhat increased; many of these, however, failed to enlarge, and the resulting clusters were straggly.

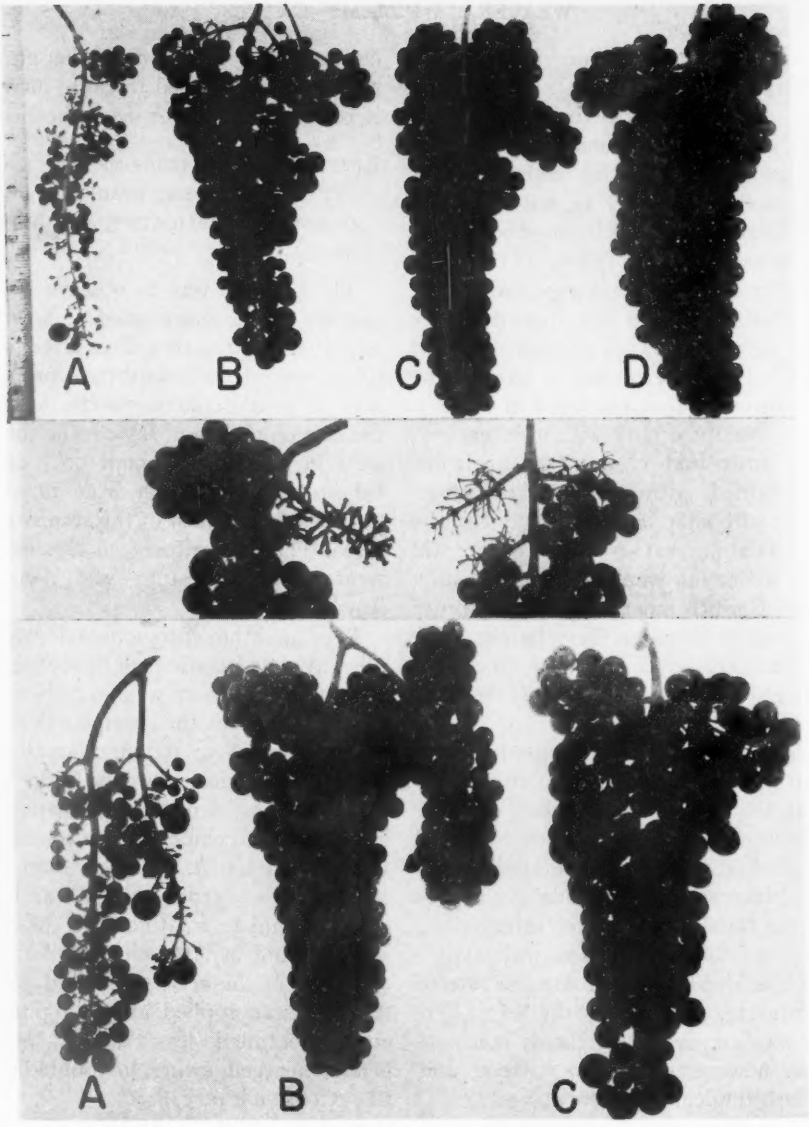
Applications of alpha-(1-naphthoxy)-propionic acid, 2,3,5-triiodobenzoic acid, N-2-chlorophenylphthalamic acid, N-1-naphthylphthalamic acid, or N-phenylphthalimide, at the concentrations indicated in table 1, resulted in well-filled clusters, but many berries contained seeds. Response of clusters to these compounds was essentially like that to 4-chlorophenoxyacetic acid. Other growth-regulators used failed to cause increases in percentage of set or size of berries.

Response of clusters of Black Corinth at different stages of development to applications of growth-regulators

The purpose was to observe the responses of clusters treated at flowering and of those treated 4 days later to applications of beta-naphthoxypropionic acid or 4-chlorophenoxyacetic acid. At the first dipping, on May 22, the clusters were in flower, and about 90% of the calyptras had fallen. On May 26, at the late treatment, most of the stamens had dried. There were fourteen clusters per treatment. Harvesting was done on August 14.

Beta-naphthoxypropionic acid was applied at concentrations of 0, 10, 50, 200, or 500 p.p.m. There was no response at either stage to the compound at 10 p.p.m., but at 50 p.p.m. a small percentage of berries enlarged. Large clusters resulted from the application on May 22 of the compound at a concentration of 200 p.p.m., but less than one-fourth of the berries enlarged and colored. A similar treatment on May 26 also resulted in large clusters, but less than half of the berries enlarged. When the acid was applied at 500 p.p.m. at either treatment, less than 5% of the berries enlarged, owing, undoubtedly, to the extensive injury.

Applications of 4-chlorophenoxyacetic acid at a concentration of 2 p.p.m. resulted in enlargement of some berries and in irregular, straggly clusters (fig. 1*B*). Large, compact clusters resulted from both early and late treatments when the acid was applied at 10, 50, or 100 p.p.m. Clusters treated with the acid at 50 or 100 p.p.m. produced very thick pedicels



FIGS. 1, 2, 3.—Clusters of Black Corinth grapes treated with 4-chlorophenoxyacetic acid. Fig. 1 (*upper*), 80 days after application made 4 days after full bloom to ungirdled vines. *Left to right:* (*A*) control; (*B*) 2 p.p.m.; (*C*) 10 p.p.m.; and (*D*) 100 p.p.m. of acid. Cluster treated with compound at 2 p.p.m. (*B*) was larger than untreated (*A*) but was irregular. Acid at two higher concentrations (*C*, *D*) resulted in large, compact clusters, and size of cluster increased with higher concentrations of acid. Fig. 2 (*middle*), 80 days after application made 4 days after full bloom with acid at 100 p.p.m. (*left*) showing thickened pedicels where berries were removed. Untreated cluster from girdled vine on right. Fig. 3 (*lower*), 83 days after spraying at full bloom. *Left to right:* (*A*) not sprayed, not girdled; (*B*) not sprayed, girdled; and (*C*) 25 p.p.m. of acid, not girdled. Note that treated cluster (*C*) has many large berries which contain seeds but that many berries did not enlarge.

(fig. 2). When the higher concentrations of 4-chlorophenoxyacetic acid used were applied, correspondingly heavier and larger clusters developed (table 2). Clusters treated with the acid 4 days after bloom were markedly heavier, with one exception, than corresponding clusters treated at time of bloom. Early application of the compound resulted in clusters with a much higher percentage of berries containing seeds than were produced from the late treatment (table 2). The majority of the berries containing seeds which resulted from application of the compound at 10 p.p.m. occurred near the tip of the rachis or tips of its branches. Since flowers near the apical ends of the rachis and its branches normally develop later, the effect of the compound upon them is probably similar to that of the early treatment. Many green berries scattered throughout the clusters treated with the acid at 50 or 100 p.p.m. at either the early or the late treatment almost invariably contained seeds.

TABLE 2

AVERAGE WEIGHT (GM.) AND PERCENTAGE OF BERRIES CONTAINING SEEDS OF BLACK CORINTH CLUSTERS TREATED WITH 4-CHLOROPHENOXYACETIC ACID. HARVESTED ON AUGUST 14

CONCENTRATION OF ACID (p.p.m.)	WEIGHT PER CLUSTER		PERCENTAGE OF BERRIES CONTAINING SEEDS	
	Treated on May 22	Treated on May 26	Treated on May 22	Treated on May 26
0	17.5	17.5	.....	.....
2	55.3	53.4	.....	6
10	97.3	140.3	23	10
50	88.4	192.0	40	13
100	123.8	196.5	100	3

A germination test with these seeds gave further indication that they were not viable. Several treated clusters containing seeds were covered with water for 3 days. By rubbing the berries on a screen the seeds were easily removed. They were dried and dropped into water. Less than 5% sank. The seeds which sank were stored in a closed bottle at about 40° F. for 6 weeks in order to break the rest period and were then placed under conditions favorable for germination. None germinated.

RESPONSE OF BLACK CORINTH VINES TO SPRAY APPLICATIONS OF GROWTH-REGULATORS

Vines of Black Corinth were sprayed with formulations of beta-naphthoxypropionic acid at concentrations of 4, 40, or 200 p.p.m., or 4-chlorophenoxyacetic acid at 1, 5, or 25 p.p.m. on May 23 when almost all calyptras had fallen from the flowers. Two vines were used per treatment. On the same date trunks of two unsprayed vines were girdled. About 20 hours previous to treatment, six clusters on each vine to be sprayed were separately inclosed in paper bags to prevent direct contact with the spray. The bags were removed when the spray had dried.

Beta-naphthoxypropionic acid at a concentration of 4 or 40 p.p.m. had no obvious effect on the foliage, but at 200 p.p.m. the compound resulted in curved tips of shoots and crinkling of young leaves within 3 days after spraying. Although applications of 4-chlorophenoxyacetic acid at 1 or 5 p.p.m. apparently produced no effect on foliage, the compound at 25 p.p.m. resulted in some curvature of tips of shoots and temporarily retarded expansion of young leaves. By harvest time, however, no injury to the foliage resulting from either of these compounds was evident.

Most vines in the entire vineyard of 40 acres showed to a slight extent typical

symptoms resulting from contact with 2,4-dichlorophenoxyacetic acid or related compounds, which probably drifted into the vineyard from outside sources. This injury may have prevented the detection of minor symptoms resulting from the growth-regulators used in the experiment.

The crop was harvested on August 9. Although clusters from vines sprayed with beta-naphthoxypropionic acid at 4 p.p.m. could not be differentiated from the untreated, those treated with the acid at 40 or 200 p.p.m. often set and enlarged approximately 25% of the berries.

Sprays of 4-chlorophenoxyacetic acid at a concentration of 1 p.p.m. were ineffective, but the compound at 5 p.p.m. resulted in some large clusters, although usually less than half the berries enlarged. Some clusters sprayed with 4-chlorophenoxyacetic acid at 25 p.p.m. were large and rather compact (fig. 3*C*, but others were straggly, as many of their berries did not enlarge. Almost half these enlarged berries contained seeds. Average weight of fruit per vine, of vines sprayed with 0, 1, 5, or 25 p.p.m. of the acid, was 1.8, 1.0, 6.3, and 17.3 pounds, respectively. The weight of fruit of the girdled but unsprayed vines was 22.4 pounds.

Clusters which were inclosed in paper bags at spraying to prevent contact with the spray did not respond to application of growth-regulators. Under the conditions of this experiment, direct contact of spray with clusters was necessary for setting and development of large berries.

Dip applications to seedless varieties other than Black Corinth

Flowering clusters of White Corinth, Black Monukka, Sultana, and Thompson Seedless were immersed in formulations of 4-chlorophenoxyacetic acid at 0.5, 5, or 50 p.p.m., or of beta-naphthoxypropionic acid at 2, 20, or 200 p.p.m.

White Corinth.—The clusters were treated on May 26, five clusters per treatment, and were harvested on August 10. Clusters and berries resulting from applications of 4-chlorophenoxyacetic acid at concentrations of 5 or 50 p.p.m. were large, whereas the untreated and those treated with the compound at 0.5 p.p.m. were relatively small (fig. 4). Other treatments with beta-naphthoxypropionic acid had no effect except for varying amounts of injury. No seeds were formed in White Corinth or in any seedless variety tested except Black Corinth.

Black Monukka.—Clusters were treated on May 25, four clusters per treatment, and were harvested on August 31. Those treated with 4-chlorophenoxyacetic acid at 5 or 50 p.p.m. were more compact and set more "shot" berries[2] than untreated clusters or those receiving the lowest concentration of the compound. The treatments with beta-naphthoxypropionic acid had no effect except to produce varying degrees of injury.

Sultana.—Lots of four clusters were used for each treatment on May 25. Harvesting was done on August 31. The setting of many "shot" berries was induced by the two higher concentrations of each of the growth-regulators. Treatment with the lowest concentration of either compound produced no apparent effects.

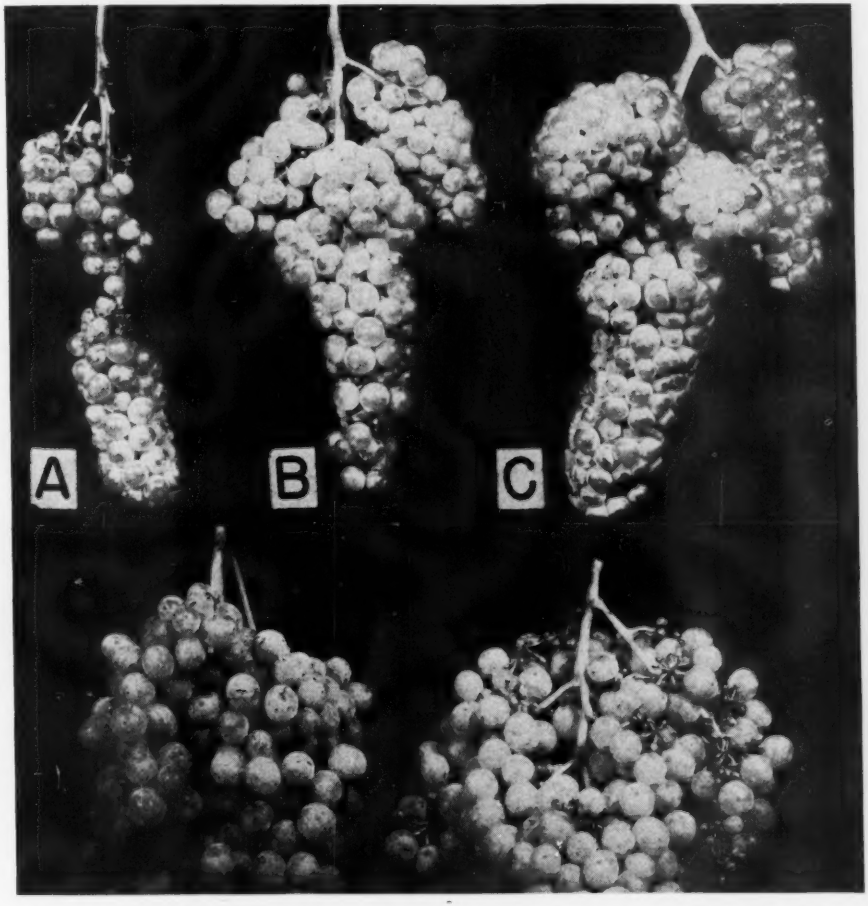
Thompson Seedless.—Lots of ten clusters were used for each treatment on May 26. At harvest on August 23 many "shot" berries adhered to clusters treated

[2] "Shot" berries refer to small, round, seedless berries that develop parthenocarpically.

with 4-chlorophenoxyacetic acid at 5 or 50 p.p.m. (fig. 5), but the compound at 0.5 p.p.m. had no apparent effect. Over half the berries which set on clusters treated with beta-naphthoxypropionic acid at 200 p.p.m. remained abnormally small, owing to the injury. Apical ends of the rachis of clusters treated with this compound at 20 p.p.m. were usually dead, but the compound at 2 p.p.m. resulted in no injury.

Spray applications of growth-regulator to Thompson Seedless vines

The purpose was to observe the response of Thompson Seedless vines to spray applications of 4-chlorophenoxy-



Figs. 4, 5.—Clusters of grapes treated with 4-chlorophenoxyacetic acid by immersion. Fig. 4 (*upper*), White Corinth 76 days after application. *Left to right:* (*A*) control; (*B*) 5 p.p.m.; and (*C*) 50 p.p.m. of acid. Note that treated clusters (*B, C*) are larger and have bigger berries than control. (Photographed August 10, 1950.) Fig. 5 (*lower*), Thompson Seedless 89 days after treatment at flowering with acid at 50 p.p.m. (*right*). Note many "shot" berries. Control on left. (Photographed August 23, 1950.)

acetic acid. Spraying was done on June 2, when most of the shatter of berries normally following the set of fruit had occurred. Earlier spraying was avoided to prevent increasing the set of "shot" berries. Concentrations of 0, 5, 20, or 50 p.p.m. of the acid were used on eight vines per treatment. At the time of treatment the trunks of seven additional vines were girdled for comparison, but these vines were not sprayed.

TABLE 3

AVERAGE WEIGHT (GM.) OF THOMPSON SEEDLESS GRAPES FROM VINES SPRAYED WITH 4-CHLOROPHENOXYACETIC ACID. HARVESTED ON AUGUST 28

Concentration of acid (p.p.m.)	Weight of 200 berries
0 (girdled)	448.4
0 (not girdled)	313.6
5 (not girdled)	392.6
20 (not girdled)	367.4
50 (not girdled)	288.4

The grapes were harvested on August 28. Berries were larger on vines sprayed with 4-chlorophenoxyacetic acid at 5 or 20 p.p.m. than on ungirdled, unsprayed vines (table 3). Pedicels on vines sprayed with the acid at 20 p.p.m. were about twice as thick as those on untreated, ungirdled vines. Foliage of vines sprayed with the compound at 20 p.p.m. was slightly bronzed, compared with that of untreated vines or of those sprayed with the acid at 5 p.p.m. Tips of some shoots sprayed with the acid at 50 p.p.m.were turning yellow 13 days after application. By August 28 entire shoots of many of these vines were yellow, and on others over half the total number of shoots were dead. Branches, especially canes completing their second season of growth, on vines sprayed with the acid at 50 p.p.m. had many calluses (fig. 6), and the canes bore only stunted shoots with injured clusters and small berries (fig. 7).

DIP APPLICATIONS TO VARIETIES CONTAINING SEEDS

MUSCAT OF ALEXANDRIA.—The purpose of this experiment was to determine whether the set of berries could be increased by using growth-regulators on this variety which usually develops clusters that are very loose and straggly.

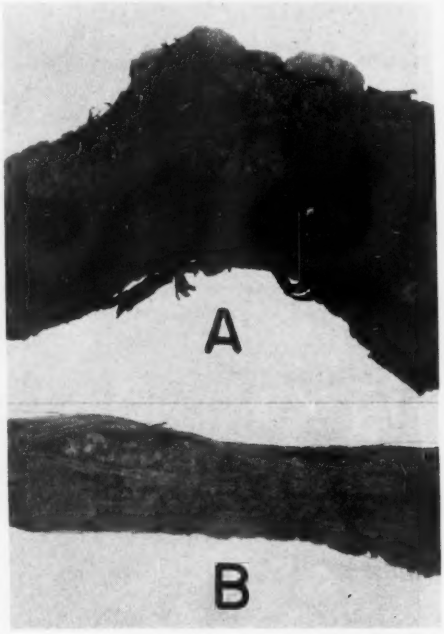
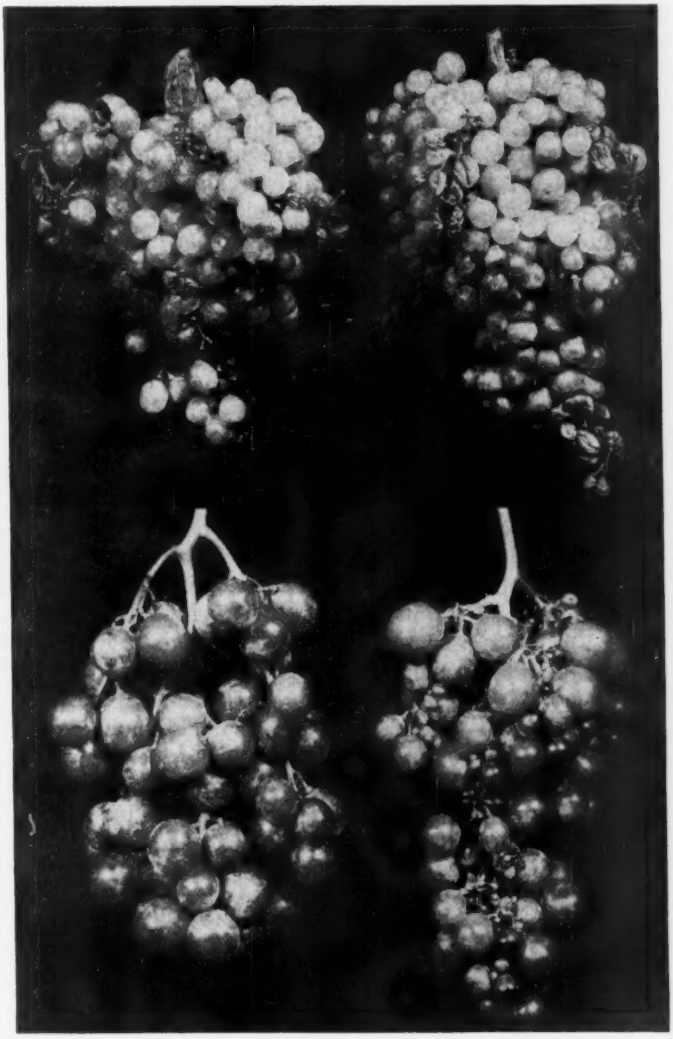


FIG. 6.—Portion of 4-year-old (*A*) and 1-year-old (*B*) branch from a Thompson Seedless vine 87 days after spraying with 4-chlorophenoxyacetic acid at 50 p.p.m. Note calluses. (Photographed September 1, 1950.)

Each lot of six flowering clusters was treated on May 25 with one of the formulations (table 1). The remaining eight or more clusters on each vine served as untreated controls.

Four days after application many

"shot" berries adhered on clusters treated with 4-chlorophenoxyacetic acid at 5 or 50 p.p.m. By harvest time, September 19, almost all "shot" berries had fallen from clusters treated with the acid at 5 p.p.m., but most of them still adhered on clusters treated with the compound at 50 p.p.m. (fig. 8). N-2-chlorophenylphthalamic acid at a concentration of 500 p.p.m. also resulted in



FIGS. 7, 8.—Clusters of grapes treated with 4-chlorophenoxyacetic acid at 50 p.p.m. Fig. 7 (*upper*), Thompson Seedless 87 days after vines were sprayed. Note injury to many berries, and splitting and callusing of peduncles. Fig. 8 (*lower*), Muscat of Alexandria 76 days after immersion (*right*). Note many small, seedless berries. Control on left. (Photographed August 9, 1950.)

the setting of many "shot" berries on some clusters, but other clusters in the same treatment were injured and failed to develop any berries of normal size.

Applications of other compounds resulted only in varying degrees of injury (table 1) with no other apparent effect. The size of these or other berries containing seeds was increased little or none by any application.

HUNISIA.—The purpose of this experiment was to attempt to produce compact clusters with large berries in this variety, which often sets many "shot" berries and sometimes has straggly clusters. Flowering clusters were immersed on May 25 (table 1). There were six clusters per treatment.

Four days after application of the higher concentrations of some of the growth-regulators, bases of many of the pedicels of berries near the apex of the rachis and its branches turned red. This discoloration of meristematic tissues was followed by much injury to the clusters (table 1). Many "shot" berries set on clusters treated with 4-chlorophenoxyacetic acid at 50 p.p.m., but by July 17 many had shattered. The other compounds did not increase the amount of set but resulted in varying degrees of injury (table 1).

Discussion

More seeds developed when 4-chlorophenoxyacetic acid was applied to clusters of Black Corinth grapes at full bloom than when applied 4 days later. Further delay in treatment might have resulted in development of berries containing no seeds. To be of commercial interest, the treatment must result in berries containing no seeds.

Applications of 4-chlorophenoxyacetic acid by dipping usually resulted in more compact clusters than did treatment with sprays. This may have been due to less effective coverage of the clusters in case of the sprays.

The development of hard seeds only in Black Corinth of the seedless varieties tested may be associated with the degree of development of the ovules and embryo sacs. PEARSON (2) has demonstrated variations in such development among the several seedless varieties.

Applications of growth-regulators often resulted in greatly increased berry size in seedless varieties but in no apparent enlargement of those containing seeds. Girdling, likewise, often greatly increases the size of seedless berries, although it has a relatively small influence on size of berries containing seeds (1).

Most berries on ungirdled, untreated vines of Black Corinth in 1950 failed to set. This shatter normally occurs in this variety. In 1949, however, the number of berries which set on ungirdled and untreated vines was often high, although the berries remained much smaller than those on girdled vines (3). Factors causing these variations in the set of fruit of Black Corinth in different seasons have not been determined. There were also variations in response to growth-regulators of clusters of Black Corinth in 1949 and 1950. For example, in 1949 the ripening of these clusters was hastened 2 weeks by applications of beta-naphthoxypropionic acid (3), but a similar application in 1950 had no apparent effect on rate of maturity.

Summary

1. In one experiment, formulations of fifteen growth-regulators were separately applied to Black Corinth grapes by dipping clusters of the flowers at full bloom. Applications of 4-chlorophenoxyacetic acid, alpha-(1-naphthoxy)-propionic acid, 2,3,5-triiodobenzoic acid, N-2-chloro-

phenylphthalamic acid, N-1-naphthylphthalamic acid, and N-phenylphthalimide resulted in some compact clusters with some large berries containing seeds.

2. In a second experiment, clusters of Black Corinth were dipped at full bloom or 4 days later into formulations of beta-naphthoxypropionic acid or 4-chlorophenoxyacetic acid. Compact clusters failed to develop after applications of the former compound, but treatment with 4-chlorophenoxyacetic acid at 10, 50, or 100 p.p.m. resulted in large, compact clusters after each treatment. With the higher concentrations of the acid used, correspondingly heavier clusters developed. A higher percentage of berries of the first treatment contained seeds than did those of the later one; clusters of the later treatment were heavier.

3. When Black Corinth vines at full bloom were sprayed with 4-chlorophenoxyacetic acid at 25 p.p.m., many large clusters developed. Average weight of fruit per vine for ungirdled but sprayed, girdled but unsprayed, and ungirdled and unsprayed was 17.3, 22.4, and 1.8 pounds, respectively.

4. Flowering clusters of White Corinth and Black Monukka grapes dipped in 4-chlorophenoxyacetic acid at 5 or 50 p.p.m. developed large compact clusters of fruit. A similar treatment with Sultana, Thompson Seedless, Muscat of Alexandria, and Hunisia induced the setting of many "shot" berries. Similar applications of other growth-regulators usually resulted in varying degrees of injury. Applications of growth-regulators to varieties containing seeds resulted in no apparent enlargement of berries.

5. Thompson Seedless vines sprayed with 4-chlorophenoxyacetic acid at 5 or 20 p.p.m. after the shatter of berries developed larger berries than did ungirdled, unsprayed vines.

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A QUANTITATIVE EVALUATION OF THE LEMNA TEST FOR HERBICIDES[1]

F. FROMM

Introduction

The practical evaluation of a potential herbicide in field tests is a time-consuming and expensive procedure. Moreover, in temperate climates it is limited to one season and is generally started only after laboratory experiments have given some evidence of the usefulness of the compound. Greenhouse tests have been used in preliminary screening. Though they need less material than field tests and are independent of outside temperatures, they are still time-consuming. The use of seed germination, or the inhibition of growth of primary roots, bean plants, or oat seedlings for an appraisal of herbicidal properties (cf. MINARIK [10] for a recent review), has been developed for testing compounds of the "growth-hormone" type. In many cases a rapid test for herbicides in general seems to be desirable. HESSENLAND and FROMM (8, 9) have used successfully the growth of duckweed, *Lemna minor*, for a quick appraisal of the weed-killing properties of halogenates. The method was later worked out in detail by GORHAM (6), OFFORD (11), and FROMM (4), using the studies of ASHBY *et al.* (1) and of CLARK and ROLLER (2) on the biology of Lemnaceae. However, the mere observation that a given substance does or does not kill duckweed is useful only to the extent that it rules out from further experimentation those compounds which have shown no effect on the test plant. Compounds lethal to duckweed must still be tested in laboratory and field experiments for their practical usefulness. Planning of such tests is limited by lack of information about the concentration of the substances to which *Lemna* is responsive which should suitably be applied in greenhouse and field tests.

[1] Paper read before the Division of Agricultural and Food Chemistry of the American Chemical Society at the meeting at Atlantic City, September 21, 1949.

Material and methods

As the first approach to this problem the action on duckweed of some well-known herbicides—sodium chlorate, ammonium thiocyanate, ammonium sulfamate, and 2,4-dichlorophenoxyacetic acid (2,4-D)—was studied quantitatively. The technique used was that described earlier by FROMM (4), but the plants were kept in 400-ml. Pyrex beakers instead of brown bottles because the difference in growth in the two kinds of containers, observed previously under the tropical conditions of Puerto Rico (4), was not confirmed under the poorer lighting at Pittsburgh. All duckweeds used were descendants of twenty plants, cultivated in Clark's solution (2) for 6 months or more. Concentration of herbicide in the solution was varied from 10^{0} to 10^{-6} molar, and the pH values from 6.5 to 6.9.

Results

Characteristic results for ammonium thiocyanate are given in figure 1. Count of number of plants showed the same trends as count of number of fronds. All

curves are drawn so that the initial number of plants (or fronds) is equal to 100%. The limiting lethal concentration seemed to be about 10^{-3} *M* and was characterized by an initial increase in the number of plants simultaneously with the inhibition of frond growth as described previously by FROMM *et al.* (5). In the same way a limiting lethal concentration of 10^{-3} *M* was found for ammonium sulfamate and of 5×10^{-3} *M* for sodium chlorate. Only the curves for frond growth in the critical range of concentration are given in figures 2 and 3 in order to simplify the presentation. Action of 2,4-D on *Lemna minor* was unexpectedly slow (figs. 4, 5). With concentrations from 10^{-3} to 10^{-5} *M* only an inhibition of frond growth but no herbicidal action was seen for the first 2 weeks. At both concentrations, however, the characteristic discrepancy between the number of plants and fronds was already established after 3 days. A concentration of 5×10^{-5} *M* may therefore be considered as that of the activity limit. OFFORD (11) reported no effect for 10 p.p.m. (equal to 10^{-4} *M* 2,4-D), but his observation was limited to a few days only and does not contradict the present findings.

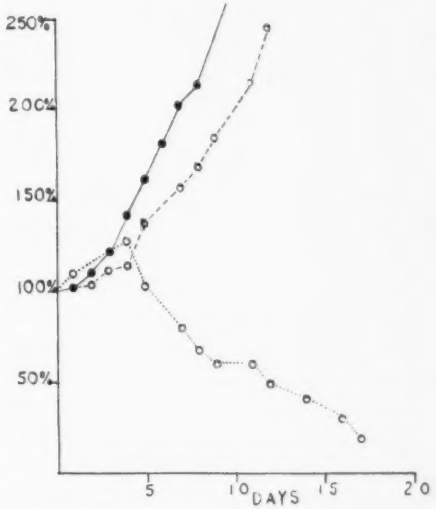
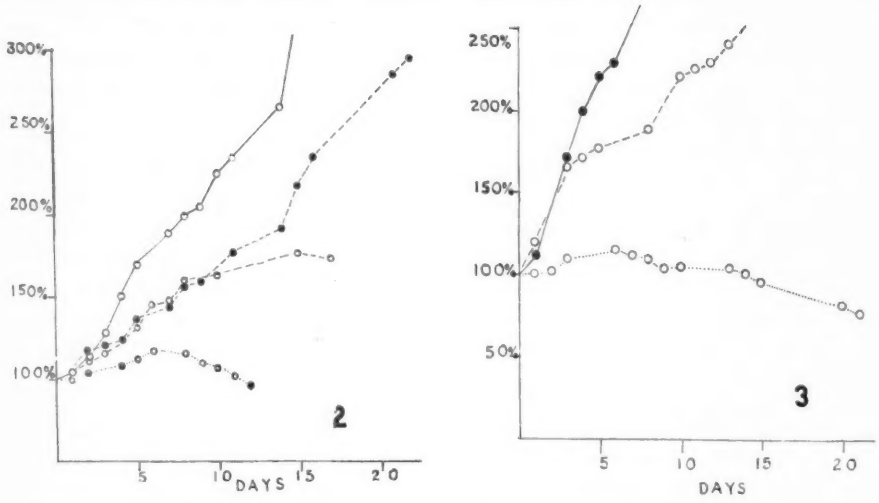


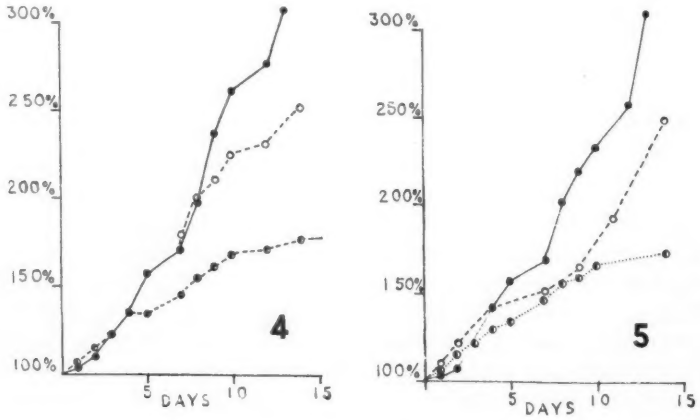
FIG. 1.—Percentage increase of *Lemna minor* plants in Clark's nutrient solution with no (*solid line*), 10^{-4} *M* (*broken line*), or 10^{-3} *M* (*dotted line*) ammonium thiocyanate added; based on number of plants at beginning of experiment as equal to 100%.

Discussion

The concentration of a herbicide applied in weed control varies widely with the species and the conditions of soil, season, form of application, etc. Practical studies of chemical weed control must furnish two measurements: the concentration of the solution and the volume of the solution applied per unit area. A review of the literature on any one of the weed-killers shows that a wide variety of units of measurement have been employed by various investigators. Concentration is given in percentage by weight, pounds per gallon, molarity, grams per liter, parts per liter, etc. Quantity per unit area is expressed in pounds per acre, gallons per acre, liters per hectare, quarts per square yard or per square rod, etc. Diversity of measurements increases unnecessarily the difficulty of comparing data of various observers. Standardization of measurements seems desirable and ought to be in agreement with international scientific practice. Hence, it is suggested that the units be standardized: the concentration in terms of molarity only, the volume in liters, and the area in square meters. These data can be expressed in the form of one fraction if the molarity is put in the numerator and the number of square meters treated with one liter of solution into the denominator. A fraction of $\frac{0.1}{2}$ will then indicate that 1 liter of 0.1 *M* solution of the agent under consideration



FIGS. 2, 3.—Growth of fronds of *Lemna minor* in Clark's nutrient solution with sodium chlorate (fig. 2) or ammonium sulfamate (fig. 3) added; based on number of fronds at beginning of experiment as equal to 100%. Fig. 2, *solid line*, control; *broken line*, *solid circles*, 10^{-4} *M* sodium chlorate; *broken line*, *open circles*, 10^{-3} *M* sodium chlorate; *dotted line*, 10^{-2} *M* sodium chlorate. Fig. 3, *solid line*, control; *broken line*, 10^{-4} *M* ammonium sulfamate; *dotted line*, 10^{-3} *M* ammonium sulfamate.



FIGS. 4, 5.—Growth of *Lemna minor* plants (fig. 4) or fronds (fig. 5) in Clark's nutrient solution with no (*solid line*), 10^{-5} *M* (*broken line*), or 10^{-4} (*dotted line*) 2,4-D added; based on number of plants (fig. 4) or fronds (fig. 5) at beginning of experiments as equal to 100%.

will control effectively the weed specified on 2 sq. m. of ground. The term "phytocidal index" is proposed for this fraction.

For the purpose of establishing a numerical relation between the limiting lethal concentration for duckweed and suitable concentrations of the weed-killer for field experiments, the phytocidal index for annual weeds in general was used. Table 1 summarizes the data for the compounds studied. The data for the phytocidal index were calculated from the literature (**3, 7, 9, 12**). As 1 liter of the solution of specified concentration was applied to 1 sq. m., the denominator of the phytocidal index becomes 1, and the phytocidal index need not be given as a fraction. In some cases it remains doubtful if the values reported in the literature represent the true phytocidal index, the lowest concentration still active on the annual weeds.

TABLE 1

RELATION BETWEEN THE LIMITING LETHAL CONCENTRATION AND PHYTOCIDAL INDEX

Compound	Limiting lethal concentration*	Phytocidal index†
$NaClO_3$	5×10^{-3}	1.9×10^{-1}
NH_4CNS	10^{-3}	2.4×10^{-1}
$NH_4O_3SNH_2$	10^{-3}	2.5×10^{-1}
2,4-D	5×10^{-5}	4.4×10^{-3}

* In moles per liter.

† Molarity if 1 liter is applied per sq. m.

If allowance is made for this uncertainty, it seems permissible to suggest that a molar concentration approximately one hundred times as high as the limiting lethal concentration is advisable for field experiments on the eradication of annual weeds. Experiments with sodium chlorate indicate that occasionally a concentration of ten to fifty times the limiting lethal concentration might also be effective, but one hundred times the limiting lethal concentration seems to be the safest interpretation. For hardy perennial weeds a higher concentration would be required. The review of literature quoted above shows that generally five to ten times the concentration used for annual weeds is applied to perennial plants. This leads to a suggestion of one thousand times the limiting lethal concentration for field experiments with perennial weeds.

Summary

1. The limiting lethal concentration for duckweed is 5×10^{-3} *M* $NaClO_3$, 10^{-3} *M* NH_4CNS or $NH_4O_3SNH_2$, and 5×10^{-5} *M* 2,4-D.

2. A "phytocidal index" is defined for weed-killers as the fraction resulting from the molarity of the active solution divided by the number of square meters which can be effectively controlled with 1 liter of this solution.

3. From examination of reported effective concentrations in the literature, the phytocidal index for annual weeds seems to be about one hundred times the limiting lethal concentration.

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THE USE OF ACTIVATED OAT SEEDS IN THE STUDY OF WINTER-HARDINESS[1]

S. S. IVANOFF

Introduction

References to the study of winter-hardiness in general and to the development of winter-hardy varieties in particular are extensive. In recent years entire volumes dealing with literature citations, abstracts, and brief reviews on winter-hardiness (**3**, **5**, **6**) have been published.

In most early work testing and selection for winter-hardiness was done principally in the field. More recently, to speed up the process, many greenhouse tests have been conducted with considerable success, using plants in pots or flats and artificial freezing in low-temperature rooms (**7**, **8**, **9**, **11**, **13**). In the following studies, use was made of activated seeds instead of plants.[2]

In such seeds the activation of the enzyme system and the subsequent speeding-up of the germination process are achieved by soaking seeds in running water for a given time and temperature. An activated seed is still in a pre-sprouting stage but is morphologically and physiologically different from a seed at rest. Its cytologic state is dynamic and sensitive, and the vital structures are more vulnerable to influences from without. The degree of activation depends upon length and temperature of soaking and on kind of seed. This new experimental approach offers the advantage of using a large mass of plant entities under conditions of easier and more exact control for the study of various aspects of winter-hardiness of both basic scientific and immediate utilitarian nature.

[1] Published as Paper no. 240, New Series, Mississippi Agricultural Experiment Station.

[2] Parts of these studies have been published in an abstract (**4**).

The primary purpose of these studies has been to find a speedy, efficient laboratory method for discovering germ plasm for resistance to cold from among large populations. To achieve this practical aim, it was necessary to study some fundamental aspects of cold-hardiness with new materials and methods.

This report deals with (*a*) the differential response to freezing of activated seeds of varieties varying in winter-hardiness, (*b*) the effect of length and temperature of soaking on survival after freezing, and (*c*) the possibilities of selection for cold-resistance from large seed populations.

Experimentation and discussion

Table 1 shows the effect of soaking and subsequent freezing of seeds of four oat varieties. Seeds were soaked in running water for 20 hours at 25° C. and then frozen for 7 days at −6°, −8°, −18°, and −30° C. Results show that (*a*) the hardier the variety, the greater the survival, and (*b*), at the lowest temperatures of freezing, some seeds of the hardy strain Forkedeer and the semi-hardy strain Delta Red 88 survived the treatments, while all seeds of the non-hardy strains Vicland and Clinton were killed. Thus it appears that the capacity of winter-hardiness exhibited by the protoplasm of activated seeds is similar to that of seedling and more mature plants as known from practical field experience.

Table 2 presents data on the effect on relative survival of various time periods of soaking and subsequent freezing at −8° C. for 3 days. In this test comparable lots of seeds of Delta Red 88 and Clinton were soaked for 0, 5, 10, 20, and 30 hours. Results show that (*a*) the longer the seeds were soaked, the greater the injury for both varieties, and (*b*) for all periods of soaking, seeds of Delta

TABLE 1

SURVIVAL OF OAT SEEDS AFTER BEING SOAKED IN RUNNING WATER FOR 20 HOURS AT 25° C. AND THEN FROZEN AT DIFFERENT TEMPERATURES*

Variety	Winter hardiness	Survival after freezing at			
		−6° C.	−8° C.	−18° C.	−30° C.
		%	%		
Forkedeer	Winter-hardy	38	16	1 in 330	1 in 1350
Delta Red 88	Semiwinter-hardy	31	10	1 in 800	1 in 7500
Vicland	Nonwinter-hardy	11	0.5	0	0
Clinton	Nonwinter-hardy	10	0	0	0

* Averages of three trials with a total of about 25,000 seeds.

TABLE 2

EFFECT OF LENGTH OF SOAKING IN RUNNING TAP WATER ON RELATIVE SURVIVAL OF OAT SEEDS, WITH SUBSEQUENT EXPOSURE TO −8° C. FOR 3 DAYS*

Length of soaking (hrs.)	Percentage survival	
	Delta Red 88 (semi-winter-hardy)	Clinton (non-winter-hardy)
0	99	97
5	42	23
10	31	5
20	11	0.5
30	8	0

* Data from three trials with four replications of 100 seeds in each treatment.

Red 88, the semihardy oat, had a higher percentage of survival than seeds of Clinton, the nonhardy oat. Here, again, activated seeds of the semihardy variety and of the nonhardy variety showed different degrees of hardiness, as do seedlings or mature plants of these varieties in the field.

In addition to greater water absorption and its effect on enzyme activities, length of soaking doubtless also influenced the amount of soluble substances extracted from the seed and had possible effects upon morphological and physiological changes within the seed.

Table 3 shows the influence upon survival after freezing of the temperature at which seeds were soaked prior to freezing. Temperature might affect not only the rate of absorption of water by the seeds but also, directly or indirectly connected with this absorption, the rate of activation of the enzyme systems of the seeds.

Seeds were soaked for 16 hours in water adjusted to 5°, 10°, 20°, 27°, 35°, and 37° C., and then frozen at −10° C. for 3 days. Results of four replicated trials showed that seeds soaked at the lowest temperature, 5°, had the highest survival, while those soaked at the highest temperature, 37° C., suffered the greatest kill. There was a pattern in the amount of killing caused by the freezing: for every 10° C. rise of soaking temperature, there was a reduction by approximately one-half in percentage of survival. Similar patterns of results were obtained when soaked seeds were frozen at −8° C. and at −12° C., but with this difference—the lower temperature gave lower values for survival, and the higher temperature gave higher values than those at −10° C. When the survival value following any soaking temperature was divided by the value at a temperature 10° C. higher, the quotient was an approximation of 2.

This pattern of values recalls the principle of VAN'T HOFF (12) relative to the effect of temperature on chemical reactions with nonelectrolytes which ARRHENIUS (1) reformulated and adapted to biological processes. CROZIER (2) and others have reported that a number of physiological processes follow ARRHENI-

TABLE 3

DEGREE OF SURVIVAL OF OAT SEEDS AT −10° C. AS RELATED TO TEMPERATURE OF WATER IN WHICH SEEDS WERE SOAKED PREVIOUS TO FREEZING*

TEMPERATURE OF SOAKING ° C.	PERCENTAGE SEEDS SURVIVING AFTER FREEZING IN TRIAL				TOTAL NO. OF SEEDS FROZEN	AVERAGE PERCENTAGE SURVIVAL AFTER FREEZING
	1	2	3	4		
5	22.0	21.0	21.4	19.3	3000	20.9
10	.....	18.3	11.5	15.6	2400	15.1
20	.....	9.6	4.4	8.3	2400	7.4
27	4.8	3.6	3.4	3.6	3000	3.9
35	2.7	1.8	1.9	1.6	3000	2.0; 2.2†
37	2.3	1.1	1.9	0.8	3000	1.5; 1.7†

* Seeds of variety Nortex were soaked for 16 hours, kept in freezing chamber for 72 hours, and germinated at room temperatures. Six to eight replications of 100 seeds each were used in each treatment.

† Values after correction made for reduction in germinability owing to high-temperature treatment before freezing.

US' equation between 0° and 40° C. and suggested that many physiological reactions catalyzed by enzymes may be the pacemakers for these processes. SIZER (10) studied the effects of temperature on the kinetics of specific enzyme reactions and attempted to apply the information to the interpretation of the effects of temperature on biological systems. The experiments reported here with freezing activated seeds seem to point to a similar parallel, namely, that different-temperature pretreatments of the seeds preceding freezing appear to determine the amount of the killing by the approximate $Q_{10} = 2$.[3]

A practical application was made of these studies with the purpose of picking out activated seeds that would survive a temperature considerably lower than germinating seeds usually encounter in the field. By using this method, from many seeds a few viable seeds could be selected which would possess germ plasm for cold resistance acquired possibly as a result of unusual hybridizations, gene rearrangements, spontaneous mutations, and other alterations of the hereditary structure of the seed.

Commercial seed in lots of 500,000 or more were activated by soaking in running water at 25°–27° C. for 20 hours; they were then divided into smaller lots of about 10,000 seeds each and frozen at −30° C. for a week or longer. They were then thawed out and allowed to germinate for several days at room temperature. Only a few seeds were able to withstand this treatment, usually in the approximate proportion of from 1 to 10,000 to 1 to 500,000. Surviving seeds were transplanted for observation and seed increase. Most of the isolated seedlings did not differ phenotypically from the variety from which they were selected. Some by their appearance, however, showed characteristics of cold-hardy individuals—more prostrate development, more narrow or twisting leaves, or more dense growth than the untreated checks of the same variety. The final test for these plants will come after their progenies are subjected to low temperatures in the field at various stages of growth.

Many of the plants thus selected may prove not to be cold-resistant. For instance, while all care is taken to soak the seeds uniformly in the running water, it is possible that an occasional seed may not absorb sufficient water to activate it. There may be some diseased seeds, the infection of which might have rendered the seed coat less permeable to water, and there may be other seeds with a naturally more impervious seed coat.

Besides selection of winter-hardy individuals, certain other results were observed following the freezing of activated seeds at low temperatures. After freezing several million seeds of nonwinter-hardy oats at −30° C., a few individuals survived which in time showed unusual features. Certain survivors developed into mature plants true to type in every

[3] Within the interval 5°–37° C., the moisture content of the 16-hour-soaked seeds increases by about 5%. The increase was in arithmetic progression and may be expressed graphically by a straight line. The moisture contents of the different parts of the seed were not determined.

Trials were also made with similarly treated seeds to determine whether the rate of germination as influenced by the different temperature treatments might be correlated with freezing injury. It was found that such correlation was not evident (although it might possibly have been obscured by another factor), since seeds soaked at 5° had about the same rate of germination as those soaked at 36°–37° C. The greatest rate of development was shown by seeds soaked between 25° and 30°. Graphically, the rate of germinaton of seeds soaked between 5° and 37° may be expressed by a parabolic curve, while the rate of killing by the freezing process may be expressed by an exponential curve.

observable respect except that they showed an extraordinary tendency toward development of buds and stems (underground and above-ground "runners"), which, in turn, developed their own roots and shoots and established themselves as separate plants after the manner of some common grasses. In another case two surviving individuals of a winter-hardy variety attained a growth period several times the ordinary life-span of an oat plant. The ordinary oat reaches maturity and production of seed within a few months after planting, loses its green color, and dies a natural death. In the case of these two cold-treated individuals, growth continued after the first crop of seed matured. Instead of death, the plants exhibited continuous and prolonged growth, producing vigorous shoots and stems and an abundance of normal seeds at all times for 12 months longer, after which their life was terminated inadvertently. The nature of these manifestations following the cold treatments needs further study.

Summary

1. Use has been made of activated oat seeds (activation of enzyme systems by soaking in running water at specified temperatures) for the study of winter-hardiness.

2. Activated seeds of winter-hardy and nonwinter-hardy oats reacted to freezing in a manner similar to seedlings or older plants of the respective varieties, as reported for field trials.

3. The temperature of the water in which seeds were soaked prior to freezing determined the percentage of survival. For every 10° C. rise in soaking temperature, there was approximately one-half reduction in survival ($Q_{10} = 2$).

4. Attempts were made to isolate cold-resistant plants in the laboratory from millions of activated seeds by freezing them at −18° to −30° C. Certain plants were isolated in connection with these studies that showed unusual characteristics, including (*a*) great extension of the life-span and (*b*) greatly increased bud development and tendency toward tillering.

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